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TITLE OF THE INVENTION (280 characters max)

A NEW ANGIOGENIC FACTOR AND ITS MEDICAL USE

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Respectfully submitted,

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PROVISIONAL APPLICATION

UNDER 37 C.F.R. § 1.53(c)

APPLICANT : HENDRIK GILLE, BEATE GAWIN, ROLF SCHÄFER, AND STEPHAN HESS

TITLE : A NEW ANGIOGENIC FACTOR AND ITS MEDICAL USE

June 10, 2003

Xantos Biomedicine AG

X62263USPRO BÖ/FLZ/bec

A new angiogenic factor and its medical use

The present invention relates to a new angiogenic factor and its use in pharmaceutical and diagnostic compositions. Furthermore, the invention relates to inhibitors of the factor and their pharmaceutical use.

Angiogenesis, the growth of new capillaries from pre-existing ones, is critical for normal physiological functions in adults [Carmeliet, P. , Mechanisms of angiogenesis and arteriogenesis. Nat Med, 2000 6 (4) 389-95]. Abnormal angiogenesis can lead to impaired wound healing, poor tissue regeneration in ischemic conditions, cyclical growth of the female reproductive system, and tumor development [Carmeliet, P. and R. K. Jain, Angiogenesis in cancer and other diseases.

Promotion of angiogenesis is desirable in situations where vascularization is to be established or extended, for example after tissue or organ transplantation, or to stimulate establishment of collateral circulation in tissue infarction or arterial stenosis. The angiogenic process is highly complex and involves the maintenance of the endothelial cells in the cell cycle, degradation of the extracellular matrix, migration and invasion of the surrounding tissue and finally, tube formation. Because of the crucial role of angiogenesis in so many physiological processes, there is a need to identify and characterize factors which will promote angiogenesis.

The administration of growth factors such as VEGF-A and FGF-2 has been considered as a possible approach for the therapeutic treatment of ischemic disorders.

[redacted]

VEGF is an endothelial cell-specific mitogen and an angiogenesis inducer that is released by a variety of tumor cells and expressed in human tumor cells in situ.

However, both animal studies and early clinical trials with VEGF angiogenesis have encountered severe problems [Carmeliet, Nat Med, 2000 6 1102-3;Yancopoulos et al., Nature. 2000 407 242-8; Veikkola et al., Semin Cancer Biol 1999 9 211-20; Dvorak et al., Semin Perinatol 2000 24 75-8; Lee et al., Circulation, 2000 102 898-901]. VEGF-A stimulated microvessels are disorganized, sinusoidal and dilated, much like those found in tumors [Lee et al., Circulation 2000 102 898-901; and Springer et al., Mol. Cell 1998 2 549-559]. Moreover, these vessels are usually leaky, poorly perfused, torturous and likely to rupture and regress. Thus, these vessels have limited ability to improve the ischemic conditions. In addition, the leakage of blood vessels induced by VEGF-A (also known as Vascular Permeability Factor) could cause cardiac oedema that leads to heart failure.

VEGF not only stimulates vascular endothelial cell proliferation, but also induces vascular permeability and angiogenesis. Angiogenesis, which involves the formation of new blood vessels from preexisting endothelium, is an important component of a variety of diseases and disorders including tumor growth and metastasis, rheumatoid arthritis, psoriasis, atherosclerosis, retinopathy, hemangiomas, immune rejection of transplanted tissues, and chronic inflammation.

In the case of tumor growth, angiogenesis appears to be crucial for the transition from hyperplasia to neoplasia, and for providing nourishment to the growing solid tumor. [Folkman, et al., Nature 339:58 (1989)]. Angiogenesis also allows tumors to be in contact with the vascular bed of the host, which may provide a route for metastasis of the tumor cells. Evidence for the role of angiogenesis in tumor metastasis is provided, for example, by studies showing a correlation between the number and density of microvessels in histologic sections of invasive human

breast carcinoma and actual presence of distant metastases. [Weidner, et al., New Engl. J. Med. 324:1 (1991)].

Expression analyses, which are shown in figure 3, show the presence of significant levels of the well known pro-angiogenic factor VEGF in tumor tissues, reflecting the above described requirement for stimulation of vascular growth into tumors, particularly solid tumors. On the other hand, the expression levels of VEGF are clearly detectable not only in malignant tissues, but also in a variety of normal cells and tissues. Consequently, the concentration of VEGF is predicted to be increased around the tissues which contain VEGF expression cells (Figure 3). This, in turn, may indicate the need not only of tumor tissue, but also of various normal tissues for VEGF mediated vascular growth. Therefore, VEGF is not a promising target when tumors, but not the surrounding tissue, are to be specifically attacked

In summary, therapeutic agents promoting revascularization with minimal toxicity are still needed and there is an ongoing requirement for new angiogenic factors and new methods of angiogenic therapy. Furthermore, there is a need for factors which specifically inhibit neovascularization in solid tumors.

The problem underlying the present invention therefore lies in providing an angiogenic agent which does not exhibit the deficiencies of VEGF as depicted above.

In the context of the present invention, it has been surprisingly found that the human protein disclosed in the NCBI database entries BAA86585, AAH44952 (see SEQ ID NO: 4) and XP_045472 (see SEQ ID NO: 6) exhibits an important role in angiogenesis both in its membrane bound form as well as in a soluble form. This protein was named SEP, and its soluble, not membrane bound form was named sSEP. The corresponding cDNA sequences are given in the NCBI database entries BC044952 and XM_045472 (SEQ ID NO: 3 and 5). Therefore, the SEP and sSEP

are a novel angiogenic factors of a to-date unknown novel family. The corresponding mouse sequences are given in SEQ ID NO: 1 (DNA) and 2 (protein)

Consequently, according to one aspect of the invention, the problem is solved by a soluble SEP (sSEP) or a functional active soluble derivative thereof.

In the context of the present invention, it could be demonstrated that SEP mediates strong angiogenic activity.

This result is totally surprising, since its sequence is not homologous to the sequence of VEGF. In Example 8, it is demonstrated that transfection of cells with DNA encoding SEP leads to the production of VEGF.

The term "sSEP" relates to any soluble SEP, wherein the amino acid sequence of SEP as demonstrated in the database has been manipulated with the consequence that the manipulated protein is soluble. In this context, sSEP relates both to artificial as well as to naturally occurring proteins. In a preferred embodiment of the invention, the sSEP of the invention does not comprise a transmembrane domain. According to Fig. 4, the transmembrane domain of SEP extends at least from amino acid 514 to amino acid 535 of the human SEP as disclosed in the data base entries AAH44952 (see SEQ ID NO: 4) and XP_045472 (see SEQ ID NO: 6). An sSEP can therefore be produced by changing the amino acid sequence in this putative transmembrane region, e.g. by exchanging hydrophobic amino acids to hydrophilic amino acids.

Methods for the production of proteins starting from a cDNA are known in the art and enclude e.g. the expression of the protein in appropriate cells or the production by subsequent addition of amino acids to a starting amino acid (Current Protocols, John Wiley & Sons, Inc., New York (2003)).

[redacted]

Furthermore, methods for the production of protein fragments are known in the art and include the cleavage of the protein with appropriate proteases or the generation of nucleic acid fragments encoding the protein fragments and subsequent expression of the fragments in appropriate cells (Current Protocols, John Wiley & Sons, Inc., New York (2003)).

Methods for the production of mutated proteins and therefore of sSEP, e.g. by exchanging one or more amino acids or by deleting stretches of amino acids, are known in the art. These methods include site directed mutagenesis of the SEP gene as disclosed in the database entries BC 044952 and XM_045472, and expressing the modified gene in appropriate cells (Current Protocols, John Wiley & Sons, Inc., New York (2003)).

The term "functional active soluble derivative" of a polypeptide within the meaning of the present invention refers to polypeptides which have a sequence homology, in particular a sequence identity, of about at least 25 %, preferably about 40 %, in particular about 60 %, especially about 70 %, even more preferred about 80 %, in particular about 90 % and most preferred of 98 % with the polypeptide. Such derivatives are e.g. the polypeptide homologous to sSEP, which originate from organisms other than the sSEP. Other examples of derivatives are polypeptides which are encoded by different alleles of the gene, of different individuals, in different organs of an organism or in different developmental phases. Functional active derivatives preferably also include naturally occurring mutations, particularly mutations that quantitatively alter the activity of the peptides encoded by these sequences. Further, such variants may preferably arise from differential splicing of the encoding genes.

In an especially preferred embodiment of the invention, the term "functional active soluble derivative" includes derivatives with single nucleotide polymorphism (SNP) at at least one of the positions 383 (G to C), 699 (A to C), 1332 (T to C),

1778 (C to T), 2260 (C to A) and/or 2896/7 (TT to GA) of the nucleotide sequence given in SEQ ID NO: 3 (BC044952).

Most preferred are SNPs at positions 383, 699 and/or 1332, leading to the amino acid exchanges E to Q, K to Q and F to S, respectively.

"Sequence identity" refers to the degree of identity (% identity) of two sequences, that in the case of polypeptides can be determined by means of for example BLASTP 2.2.5 and in the case of nucleic acids by means of for example BLASTN 2.2.6, wherein the low complexity filter is set on and BLOSUM is 62 (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402).

"Sequence homology" refers to the similarity (% positives) of two polypeptide sequences determined by means of for example BLASTP 2.0.1 wherein the Filter is set on and BLOSUM is 62 (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402).

Nucleic acids encoding functional active derivatives can be isolated by using human SEP gene sequences in order to identify homologues with methods known to a person skilled in the art, e.g. through PCR amplification or hybridization under stringent conditions (e.g. 60 °C in 2.5 x SSC buffer followed by several washing steps at room temperatureer concentration) with suitable probes derived from e.g. the human SEP sequences according to standard laboratory methods.

"Functional active derivative" refers to a polypeptide that has essentially the biological function(s) as the corresponding protein. In the case of sSEP, this may be an angiogenic activity as demonstrated in Examples 2 and 3. A test for the determination of the angiogenic activity of a putative sSEP derivative is demonstrated in Example 2.

[redacted]

Furthermore, in case of sSEP, the same biological activity may also be the ability to compete with membrane bound SEP and therefore to act as an inhibitor of a signal transduced by membrane bound SEP.

In the case of membrane bound SEP, the term "Functional active derivative" may refer to the ability to induce the expression of VEGF as shown in Example 8.

According to a preferred embodiment of the invention, the sSEP or functional derivative thereof of the invention is devoid of a transmembrane domain of SEP or of functional active variant thereof. Preferably, this means that a C-terminal fragment containing the transmembrane domain of the SEP or of the functional active derivative thereof has been cleaved off. More preferably, also a N-terminal fragment has been cleaved off. Preferably, sSEP fragments are produced by cleaving at potential protease cleaving sites, more peferably at the following potential cleaving sites:

SPRAIPRN (amino acids 165 to 172 of SEP as given in SEQ ID NO: 4)
ARSTPRASRL (amino acids 242 to 250 of SEP as given in SEQ ID NO: 4)
HRPSP (amino acids 509 to 513 of SEP as given in SEQ ID NO: 4)

Cleaving can occur within every amino acid within these sequences, however, a cleaving after the amino acid R is preferred.

According to the invention, this includes also that after cleavage with an appropriate protease, further amino acids are removed by e.g. carboxypeptidases.

Consequently, in a more preferred embodiment, the sSEP or functional derivative thereof of the invention has a C-terminal amino acid corresponding to amino acid 510, 249, 246, 242, 171 or 167 of SEP according to SEQ ID NO: 4 or has a C-terminal amino acid corresponding to the equivalent amino acid of a SEP derivative.

In a most preferred embodiment, an sSEP according to the invention has one of the sequences as shown in Figure 5 (SEQ ID NO: 7-17).

Within the invention it is also included that, in case that a fragment of the invention still comprises a signal peptide, this signal peptide may also be cleaved off.

As demonstrated first in the context of the present invention, the protein depicted in SEQ ID NO: 2, 4 or 6 and soluble variants thereof exhibit an important role in angiogenesis. This enables the use of these proteins in therapy.

Consequently, the invention further relates to a pharmaceutical composition comprising

a) the sSEP or derivative thereof of the invention,
b) SEP as defined in SEQ ID NO: 2, 4 or 6,
c) a functional active derivative of the SEP of section b), and/or
d) a nucleic acid encoding the proteins of sections a), b) or c) above,

optionally in combination with a pharmaceutically acceptable carrier.

The molecules as depicted in sections a) to d) may be provided as defined above.

Examples of nucleic acids as defined in d) are the nucleic acids shown in SEQ ID NO: 1, 3, and 5. Other examples are nucleic acids encoding the derivatives and fragments as described above.

In a preferred embodiment of the invention, the pharmaceutical composition further comprises VEGF, and/or a functional derivative thereof, perferably in combination with VEGF-A, VEGF-B, VEGF-C, VEGF-D, PLGF, PDGF-A, PDGF- B, PDGF-C, PDGF-D and FGF.

As already mentioned above, VEGF is a well known angiogenic factor. Consequently, a combination of SEP and VEGF leads to enforced or synergistic effects in the promotion of angiogenesis in mammals.

The invention also relates to the sSEP or derivative thereof of the invention or a SEP as defined in SEQ ID NO: 2, 4 or 6 or functional active derivates thereof or of nucleic acids encoding these molecules for use in therapy.

The pharmaceutical composition of the invention may be applied as follows:

In accordance with the invention, there are numerous techniques which can be used to administer an effective vascuologenesis promoting or angiogenesis stimulating amount of SEP, sSEP or a functional active derivative thereof to a patient suffering from ischemia or some other condition which may be alleviated by vasculogenesis or angiogenesis. SEP administration may be effected either as recombinant protein or by gene transfer either as naked DNA or in a vector [Kornowski R,Fuchs S, Leon MB, Epstein SE, Delivery strategies to achieve therapeutic myocardial angiogenesis, Circulation, 2000 101 (4) 454-8; Simons M, Bonow RO, Chronos NA, Cohen DJ, Giordano FJ, Hammond HK, et al., Clinical trials in coronary angiogenesis: issues, problems, consensus: An expert panel summary, Circulation, 2000 102 (11) E73-86; and Isner JM, Asahara T, Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization, J Clin Invest, 1999 103 (9) 1231-36].

If desired, regulatable vectors may be used as described in Ozawa et al, Annu Rev Pharmacol. & Toxicol, 2000 40 295-317. For example, SEP or sSEP can be administered by direct myocardial injection of naked plasmid DNA encoding SEP, sSEP or a functional active derivative thereof during surgery in patients with chronic myocardial ischemia following procedures outlined in Vale, P. R., et al., Left ventricular electromechanical mapping to assess efficacy of phVEGF (165)

gene transfer for therapeutic angiogenesis in chronic myocardial ischemia, Circulation, 2000 102 965-74. SEP, sSEP or a functional active derivative thereof can also be administered by direct myocardial injection of SEP, sSEP or a functional active derivative thereof protein via a minithoracotomy. Preferably, it is given as a bolus dose of from 1 pg/kg to 15 mg/kg, preferably between 5 pg/kg and 5 mg/kg, and most preferably between 0.2 and 2 mg/kg. Continuous infusion may also be used, for example, by means of an osmotic minipump as described in Heyman et al., Nat Med, 1999 5 1135-152. If so, the medicament may be infused at a dose between 5 and 20 ug/kg/minute, preferably between 7 and 15 pg/kg/minute.

Alternatively SEP, sSEP or a functional active derivative thereof can be administered by catheterbased myocardial SEP, sSEP or a functional active derivative thereof gene transfer. In this technique, a steerable, deflectable 8F catheter incorporating a 27guage needle is advanced percutaneously to the left ventricular myocardium. A total dose of 200 ug/kg is administered as 6 injections into the ischemic myocardium (total, 6. 0 mL). Injections are guided by NOGA left ventricular electromechanical mapping. See Vale, P. R., et al., Randomized, single-blind, placebo-controlled pilot study of catheter-based myocardial gene transfer for therapeutic angiogenesis using left ventricular electromechanical mapping in patients with chronic myocardial ischemia, Circulation, 2001 103 (17) 2138-43.

Another possibility for SEP, sSEP or a functional active derivative thereof administration is injection of SEP plasmid in the muscles of an ischemic limb in accordance with procedures described in Simovic, D., et al., Improvement in chronic ischemic neuropathy after intramuscular phVEGF165 gene transfer in patients with critical limb ischemia, Arch Neurol, 2001 58 (5) 76168.

Still another technique for effective administration is by intra-arterial gene transfer of the gene using adenovirus and replication defective retroviruses as described for VEGF in Baumgartner I and Isner JM, Somatic gene therapy in the cardiovascular system, Annu. Rev Physiol, 2001 63 427-50. An additional possi-

bility for administering SEP, sSEP or a functional active derivative thereof is by intracoronary and intravenous administration of recombinant SEP, sSEP or a functional active derivative thereof following procedures described in Post, M. J., et al., Therapeutic angiogenesis in cardiology using protein formulations, Cardiovasc Res, 2001 49 522-31.

A still further possibility is to use ex vivo expanded endothelial progenitor cells (EPCs) engineered to express SEP, sSEP or a functional active derivative thereof for myocardial neovascularization as described in Kawamoto, A., et al., Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation, 2001 103 (5) 634-37.

Yet another technique which may be used to administer SEP, sSEP or a functional active derivative thereof is percutaneous adenovirus-mediated gene delivery to the arterial wall in injured atheroinatous stented arteries. See, for example, Maillard, L., et al., Effect of percutaneous adenovirus-mediated Gax gene delivery to the arterial wall in double-injured 'atheromatous stented rabbit iliac arteries, Gene Ther, 2000 7 (16) 1353-61 ; and Laham RJ, Simons M, and Sellke F, Gene transfer for angiogenesis in coronary artery disease, Annu Rev Med, 2001 52 485-502.

In one advantageous aspect of the invention, a therapeutically effective dose of SEP, sSEP or a functional active derivative thereof is administered by bolus injection of the active substance into ischemic tissue, e. g. heart or peripheral muscle tissue. The effective dose will vary depending on the weight and condition of the ischemic subject and the nature of the ischemic condition to be treated. It is considered to be within the skill of the art to determine the appropriate dosage for a given subject and condition. Furthermore, the pharmaceutical composition can be administered in further conventional manners, e.g. by means of the mucous membranes, for example the nose or the oral cavity, in the form of dispositories implanted under the skin, by means of injections, infusions or gels which contain the medicaments according to the invention. It is further possible to administer the

medicament topically and locally, if appropriate, in the form of liposome complexes. Furthermore, the treatment can be carried out by means of a transdermal therapeutic system (TTS), which makes possible a temporally controlled release of the medicaments. TTS are known for example, from EP 0 944 398 A1, EP 0 916 336 A1, EP 0 889 723 A1 or EP 0 852 493 A1.

In accordance with another aspect of the invention, SEP, sSEP or a functional active derivative thereof is administered by continuous delivery, e. g., using an osmotic minipump, until the patient is able to selfmaintain a functional vasculer network.

In another advantageous aspect within the scope of the invention, SEP, sSEP or a functional active derivative thereof is effectively administered to an ischemic subject by contacting ischemic tissue with a viral vector, e. g. an adenovirus vector, containing a polynucleotide sequence encoding the protein operatively linked to a promoter sequence.

SEP, sSEP or a functional active derivative thereof may also be effectively administered by implantation of a micropellet impregnated with active substance in the direct vicinity of ischemic tissue.

For the production of the pharmaceutical compositions of the invention, the molecules of the present invention are usually formulated with suitable additives or auxiliary substances, such as physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease inhibitors, preferably aprotinin, e-aminocaproic acid or pepstatin A or sequestering agents such as EDTA, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc. depending on the kind of administration.

Suitable further additives are, for example, detergents, such as, for example, Triton X-100 or sodium deoxycholate, but also polyols, such as, for example, poly-

[redacted]

ethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic compounds, such as, for example, amino acids such as glycine or in particular taurine or betaine and/or a protein, such as, for example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

The physiological buffer solution preferably has a pH of approx. 6.0-8.0, especially a pH of approx. 6.8-7.8, in particular a pH of approx. 7.4, and/or an osmolarity of approx. 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pH of the medicament is in general adjusted using a suitable organic or inorganic buffer, such as, for example, preferably using a phosphate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4-(2-hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3-morpholino-1-propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

Injection solutions are in general used if only relatively small amounts of a solution or suspension, for example about 1 to about 20 ml, are to be administered to the body. Infusion solutions are in general used if a larger amount of a solution or suspension, for example one or more litres, are to be administered. Since, in contrast to the infusion solution, only a few millilitres are administered in the case of injection solutions, small differences from the pH and from the osmotic pressure of the blood or the tissue fluid in the injection do not make themselves noticeable or only make themselves noticeable to an insignificant extent with respect to pain sensation. Dilution of the formulation according to the invention before use is therefore in general not necessary. In the case of the administration of relatively large amounts, however, the formulation according to the invention should be diluted briefly before administration to such an extent that an at least approximately isotonic solution is obtained. An example of an isotonic solution is a 0.9% strength sodium chloride solution. In the case of infusion, the dilution can be car-

[redacted]

ried out, for example, using sterile water while the administration can be carried out, for example, via a so-called bypass.

Within the present invention, subjects which may be treated or diagnosed include animals, preferably mammals and humans, dead or alive. These patients suffer from the diseases as mentioned above.

Furthermore, the invention relates to the use of the sSEP or derivative thereof of the invention or of the SEP as defined in SEQ ID NO: 2, 4 or 6 or a functional active derivative thereof or of a nucleic acid encoding these molecules for the preparation for the treatment of ischemic, dental or placental diseases, of smoker's leg, of diabetic ulcers or for the stimulation of wound healing, especially of wound healing of fractures.

These diseases are all characterised by a disturbed angiogenesis and therefore SEP, either as a soluble factor or as defined in SEQ ID NO: 2, 4 or 6 as well as functional active derivatives thereof lead to a significant improvement in these diseases.

With respect to the wound healing of fractures, SEP immobilised to a matrix can be administered directly into the site of fracture to promote the angiogenesis and wound healing. As matrices can be used ceramic matrices or bonemeal on which the protein is immobilised. Slow release formulations to have the factor locally enriched can be used as well.

With respect to the treatment of placental diseases, neovascularization is an essential requirement for supporting the growing fetus and embryo during pregnancy. For that process, vascular development is necessary in the placenta (fetal as well as maternal tissue) as well as in the uterus. Expression analyses, which are shown in Figure 3, show the presence of significant levels of VEGF in uterus, reflecting the above described requirement for stimulation of vascular growth in this tissue.

On the other hand, compared with placenta, the expression levels of VEGF are relatively low in placenta. Thus, the limited expression of VEGF in placenta may - by itself - not be sufficient to stimulate sufficient vascularization. The high expression of SEP in female placenta, as shown in figure 3, provides an explanation for the lower levels of VEGF expression in placenta compared to uterus. SEP is highly expressed in normal placenta but is found at reduced levels in human uterus. Thus, vascularization in uterus appears to be predominantly stimulated by VEGF, while in placenta, SEP may play a more pronounced function. Hereby, both factors, each with defined specificity, are complementing their function to stimulate vascularization. In consequence, both factors are necessary for sufficient vascularization during pregnancy.

Because of that, deficiencies in SEP may cause infertility, problems in pregnancy. Consequently, supplementation of SEP may aid to ameliorate or prevent said disorders. Furthermore, inhibition of SEP may be used to prevent angiogenesis in early pregnancies, with the objective to terminate pregnancies in humans (or animals) due to medical indications.

In a preferred embodiment of the invention, sSEP, SEP or functional active derivatives thereof are used in combination with VEGF and/or functional active derivatives thereof, perferably in combination with VEGF-A, VEGF-B, VEGF-C, VEGF-D, PLGF, PDGF-A, PDGF- B, PDGF-C, PDGF-D and FGF.

The invention further includes a method for the treatment of a patient in need of such treatment, wherein an effective amount of SEP, sSEP or a functional active derivative thereof is administered to the patient.

With respect to the preparation of this pharmaceutical composition, its administration and other embodiments the same apply as defined above.

As it is shown in the examples, SEP is especially upregulated in several tumor diseases. Consequently, SEP, sSEP and functional active derivatives thereof can be used as diagnostic agents.

The invention therefore relates to a diagnostic agent comprising

a) the sSEP or derivative thereof of the invention
b) SEP as defined in SEQ ID NO: 2, 4 or 6,
c) a functional active derivative of the SEP of section c),
d) a nucleic acid encoding the SEPs of sections a) to c), and/or
e) means for detecting the proteins of sections a) to c) or the nucleic acids of section d).

This diagnostic agent may be appropriately combined with additional carriers or diluents or other additives which are suitable in this context. With respect to these agents, the same apply as defined above for the pharmaceutical composition of the invention.

Furthermore, the invention relates to the sSEP or derivatives thereof of the invention, SEP as defined in SEQ ID NO: 2, 4 or 6, a functional active derivative thereof, a nucleic acid encoding these SEPs or functional active derivatives and/or of means for detecting these SEPs or nucleic acids for use in diagnosis.

The proteins or nucleic acids may be prepared as defined above.

Within the meaning of the present invention, means of detecting the proteins of the invention or SEP or functional active derivatives thereof include antibodies which can e.g. applied in Westen Blotting, Immunohistochemistry, ELISA or functional assays for the proteins (Current Protocols, John Wiley & Sons, Inc. (2003)).

Means for detecting the nucleic acids as defined above include other nucleic acids being capable of hybridizing with the nucleic acids e.g. in Southern Blots or Northern Blots as well as during In Situ Hybridization (Current Protocols, John Wiley & Sons, Inc. (2003)).

Furthermore, the invention relates to the use of the sSEP or derivatives of the invention or of SEP as defined in SEQ ID NO: 2, 4 or 6 or functional active derivatives thereof, of a nucleic acid encoding these SEPs or derivatives thereof or of means for detecting the SEPs or nucleic acids above for the diagnosis of tumor or tumor progression.

SEP is an important marker of tumor cells (as shown in Fig. 3). Angiogenesis is generally a phenomenon which occurs in later tumor stages. Therefore, SEP represents a marker for later tumor stages, i.e. for tumors which have already achieved a malignant state.

For example, sSEP or functional active derivatives thereof may be detected in the serum via antibodies. Furthermore, SEP, sSEP or functional active derivatives thereof may be detected in the tumor tissue via immunohistochemistry. Nucleic acids encoding these molecules, e.g. mRNA, may be detected using quantitative PCR.

Depending of the tumor progression and of the occurrence of a tumor, sSEP expression in the serum may change. Consequently, by measuring serum levels, it can be determined whether a patient is susceptible for an SEP or sSEP mediated tumor therapy. The higher the SEP or sSEP expression, the better a therapeutical success can be predicted.

In several diseases as mentioned below, an aberrant angiogenesis contributes the clinical symptoms or is even the reason for these symptoms. The present invention relates to SEP, which is an important inducer of angiogenesis, e.g. in tumors.

In contrast to VEGF, the expression of SEP is predominantly restricted to tumor cells. Especially the expression of SEP in uterus appears to fulfil a defined biological function, as described further in figure 3. The rather specific expression of SEP in cancerous tissues makes SEP a valuable target for cancer therapy. Consequently, the inhibition of SEP results in inhibition of angiogenesis which will result in the treatment of these diseases. Because of the greater tumor-vs-normal specificity of SEP, said inhibitory substances have an increased tumor specificity.

In another aspect of the invention, the invention therefore also relates to an inhibitor of the sSEP or derivatives thereof of the invention or of the SEP as defined in SEQ ID NO: 2, 4 or 6 or of functional active derivatives thereof.

According to the present invention the term "inhibitor" refers to a biochemical or chemical compound which preferably inhibits or reduces the angiogenic activity of sSEP, SEP or the derivatives thereof. This can e.g. occur via suppression of the expression of the corresponding gene. The expression of the gene can be measured by RT-PCR or Western blot analysis.

Examples of such SEP inhibitors are binding proteins or binding peptides directed against SEP, in particular against the active site of SEP, and nucleic acids directed against the SEP gene

In a preferred embodiment, the inhibitor of the invention is selected from the group consisting of antibodies, antisense oligonucleotides, siRNA, low molecular weight molecules (LMWs) and SEP receptor antagonists.

LMWs are molecules which are not proteins, peptides antibodies or nucleic acids, and which exhibit a molecular weight of less than 5000 Da, preferably less than 2000 Da, more preferably less than 2000 Da, most preferably less than 500 Da. Such LMWs may be identified in High-Through-Put procedures starting from libraries. Such methods are known in the art.

The term "binding protein" or "binding peptide" refers to a class of proteins or peptides which bind and inhibit sSEP, SEP or derivatives thereof including, without limitation, polyclonal or monoclonal antibodies, antibody fragments and protein scaffolds directed against these proteins.

The procedure for preparing an antibody or antibody fragment is effected in accordance with methods which are well known to the skilled person, e.g. by immunizing a mammal, for example a rabbit, with sSEP, SEP or derivatives thereof, where appropriate in the presence of, for example, Freund's adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al. (1981) The New England Journal of Medicine: 1344-1349). The polyclonal antibodies which are formed in the animal as a result of an immunological reaction can subsequently be isolated from the blood using well known methods and, for example, purified by means of column chromato-graphy. Monoclonal antibodies can, for example, be prepared in accordance with the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293-299).

According to the present invention the term antibody or antibody fragment is also understood as meaning antibodies or antigen-binding parts thereof, which have been prepared recombinantly and, where appropriate, modified, such as chimaeric antibodies, humanized antibodies, multifunctional antibodies, bispecific or oligospecific antibodies, single-stranded antibodies and F(ab) or $F(ab)_2$ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213 or WO 98/24884).

As an alternative to the classical antibodies it is also possible, for example, to use protein scaffolds against sSEP, SEP or derivatives thereof, e.g. anticalins which are based on lipocalin (Beste et al. (1999) Proc. Natl. Acad. Sci. USA, 96, 1898-1903). The natural ligand-binding sites of the lipocalins, for example the retinol-binding protein or the bilin-binding protein, can be altered, for example by means

of a "combinatorial protein design" approach, in such a way that they bind to selected haptens, here to sSEP, SEP or derivatives thereof (Skerra, 2000, Biochim. Biophys. Acta, 1482, 337-50). Other known protein scaffolds are known as being alternatives to antibodies for molecular recognition (Skerra (2000) J. Mol. Recognit., 13, 167-187).

If it is intended to inhibit the functions of membrane bound SEP, also sSEP may be an inhibitor of the invention, since sSEP may compete with SEP for the bindung of SEP to its receptor or ligand.

The term "nucleic acids against the SEP gene or SEP itself" refers to double-stranded or single stranded DNA or RNA which, for example, inhibit the expression of the SEP gene or the activity of sSEP, SEP or derivatives thereof and includes, without limitation, antisense nucleic acids, aptamers, siRNAs (small interfering RNAs) and ribozymes.

The nucleic acids, e.g. the antisense nucleic acids or siRNAs, can be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584). Aptamers are nucleic acids which bind with high affinity to a polypeptide, here sSEP, SEP or derivatives thereof. Aptamers can be isolated by selection methods such as SELEX (see e.g. Jayasena (1999) Clin. Chem., 45, 1628-50; Klug and Famulok (1994) M. Mol. Biol. Rep., 20, 97-107; US 5,582,981) from a large pool of different single-stranded RNA molecules. Aptamers can also be synthesized and selected in their mirror-image form, for example as the L-ribonucleotide (Nolte et al. (1996) Nat. Biotechnol., 14, 1116-9; Klussmann et al. (1996) Nat. Biotechnol., 14, 1112-5). Forms which have been isolated in this way enjoy the advantage that they are not degraded by naturally occurring ribonucleases and, therefore, possess greater stability.

Nucleic acids may be degraded by endonucleases or exonucleases, in particular by DNases and RNases which can be found in the cell. It is, therefore, advantageous to modify the nucleic acids in order to stabilize them against degradation, thereby ensuring that a high concentration of the nucleic acid is maintained in the cell over a long period of time (Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Typically, such a stabilization can be obtained by introducing one or more internucleotide phosphorus groups or by introducing one or more non-phosphorus internucleotides.

Suitable modified internucleotides are compiled in Uhlmann and Peyman (1990), supra (see also Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid which can be employed in one of the uses according to the invention contain, for example, methyl phosphonate, phosphorothioate, phosphoramidate, phosphorodithioate and/or phosphate esters, whereas non-phosphorus internucleotide analogues contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also the intention that this modification should improve the durability of a pharmaceutical composition which can be employed in one of the uses according to the invention.

The use of suitable antisense nucleic acids is further described e.g. in Zheng and Kemeny (1995) Clin. Exp. Immunol., 100, 380-2; Nellen and Lichtenstein (1993) Trends Biochem. Sci., 18, 419-23, Stein (1992) Leukemia, 6, 697-74 or Yacyshyn, B. R. et al. (1998) Gastroenterology, 114, 1142).

The production and use of siRNAs as tools for RNA interference in the process to down regulate or to switch off gene expression, here SEP gene expression, is e.g. described in Elbashir, S. M. et al. (2001) Genes Dev., 15, 188 or Elbashir, S. M. et al. (2001) Nature, 411, 494. Preferably, siRNAs exhibit a length of less than 30

nucleotides, wherein the identity stretch of the sense strang of the siRNA is preferably at least 19 nucleotides.

Ribozymes are also suitable tools to inhibit the translation of nucleic acids, here the SEP gene, because they are able to specifically bind and cut the mRNAs. They are e.g. described in Amarzguioui et al. (1998) Cell. Mol. Life Sci., 54, 1175-202; Vaish et al. (1998) Nucleic Acids Res., 26, 5237-42; Persidis (1997) Nat. Biotechnol., 15, 921-2 or Couture and Stinchcomb (1996) Trends Genet., 12, 510-5.

Thus, the nucleic acids described can be used to inhibit or reduce the expression of the SEP genes in the cells both in vivo and in vitro and consequently act as a SEP inhibitor in the sense of the present invention. A single-stranded DNA or RNA is preferred for the use as an antisense oligonucleotide or ribozyme, respectively.

The invention further relates to a pharmaceutical composition, comprising the inhibitor of the invention, optionally in combination with a pharmaceutically acceptable carrier. With respect to the preparation and administration of this pharmaceutical composition of the invention, the same applies as defined above for other pharmaceutical compositions of the invention.

In a preferred embodiment, this pharmaceutical composition of the invention further comprises a VEGF inhibitor.

Another aspect of the invention relates to the inhibitor of the invention for use in therapy.

The invention further relates to the use of an inhibitor of the invention for the preparation of a pharmaceutical composition for the treatment of cancer, rheumatoid arthritis, psoriasis, artherosclerosis, retinopathy, osteoarthritis, endometriosis and chronic inflammation.

For the context of these diseases, SEP inhibition aims at preventing the formation of vascular vessels which support the diseased tissue. This, in turn, will reduce the amount of diseased or malignant cells (e.g. cancer cells).

According to a preferred embodiment, the cancer is selected from the group consisting of brain cancer, pancreas carcinoma, stomach cancer, colon carcinoma, skin cancer, especially melanoma, bone cancer, kidney carcinoma, liver cancer, lung carcinoma, ovary cancer, mamma carcinoma, uterus carcinoma, prostate cancer and testis carcinoma.

The invention further includes a method for the treatment of a patient in need of such treatment, wherein an effective amount of an inhibitor of SEP, sSEP or of a functional active derivative thereof is administered to the patient.

With respect to the preparation of this pharmaceutical composition, its administration and other embodiments the same apply as defined above.

Preferably, the inhibitor is used in combination with a VEGF and inhibitor. In this case, the definition of an inhibitor is as mentioned above, only in the context of VEGF and not SEP.

The invention further relates to a method for the identification of a SEP inhibitor, wherein a potential inhibitor is tested for its activity to block the effects of SEP, sSEP or of a functional derivative thereof.

In this method of the invention, in general, SEP, sSEP or the corresponding gene are provided e.g. in an assay system and brought directly or indirectly into contact with a test compound, in particular a biochemical or chemical test compound. Then, the influence of the test compound on SEP, sSEP or the corresponding gene is measured or detected by measuring whether the SEP phenotype is reversed by

addition of the potential inhibitor. Thereafter, suitable inhibitors can be analyzed and/or isolated. For the screening of compound libraries, the use of high-throughput assays are preferred which are known to the skilled person or which are commercially available.

Suitable assays may be based on the gene expression of SEP or sSEP or on the physiological activity of SEP or sSEP, i.e. the angiogenic properties.

For example, the following assay may be used for the identification of an inhibotor of the invention:

- transfection of SEP, mSEP (murine SEP; Xantos clone collection), hSEP (human SEP; received by RZPD clone services) into HEK293 cells
- transfer of supernatants of HEK 293 cells onto HUVEC cells (as described for the screen in example I)
- addition / incubation of HUVEC cells with LMW (low molecular weight) compound library or other potential inhibitors
- screening for inhibition of proliferating activity (reversion of phenotype)
- definition of lead structures
- analysis of specificity: inhibition of SEP, no effect on VEGF

The experimental steps transfection of 293 cells, transfer of supernatant onto HUVEC cells and screening for proliferation or inhibition of proliferation, repectively, can be carried out according to examples 1 and 2.

The invention further relates to a method for the preparation of a pharmaceutical composition, wherein a SEP inhibitor is identified as indicated above, synthesized in adequate amounts and finally formulated into a pharmaceutical composition.

Furthermore, the invention relates to the identification of SEP interacting proteins, e.g. receptors or pathway components, wherein

a) a potential SEP interactor is brought into contact with SEP or a functional derivative thereof and

b) binding of the potential interactor to SEP or the functional derivative thereof is determined.

An example for different strategies for providing an interactor of SEP is given in Example 6.

The following Figures and Examples are intend to illustrate further the invention without limiting it.

Short Description of the Figures:

Figure 1:

Proliferation of HUVEC following transfer of supernatants from transfected 293 cells.

The relative fluorescence units (RFU) are given as mean value from three independent experiments. Experiments were performed following the manually adapted protocol described above. Vector represents the negative control resulting from transfection of the cloning vector pCMV-Sport6 into 293 cells and measurement of Alamar Blue to determine background proliferative effect of the supernatant derived from 293 cells. VEGF and PDGF were derived from the same clone collection to ensure compatibility of expression systems.

Figure 2:

Proliferation of NHDF (normal human dermal fibroblasts) following transfer of supernatants from transfected 293 cells.

The relative fluorescence units (RFU) are given as mean value from three independent experiments.

Experiments were performed following the manually adapted protocol described above

Vector represents the negative control resulting from transfection of the cloning vector pCMV-Sport6 into 293 cells and measurement of Alamar Blue to determine background proliferative effect of the supernatant derived from 293 cells.

VEGF and PDGF were derived from the same clone collection to ensure compatibility of expression systems.

The results shown in Fig. 1 and 2 demonstrate that SEP acts specifically on endothelial but not on fibroblast cells

Figure 3:

Increased expression of SEP in tumor vs normal tissue and comparison to VEGF of tumor vs normal specificity

Database analyses reveales the frequencies of EST'hits' in public databases (NCBI CGAP, 5-16-03), which are indicative for relative expression levels in various normal and malignant tissues. Shown are normalized 'hit' frequencies per 200.000 EST entries x library. Note the different expression pattern in normal tissue (VEGF predominantly in uterus, SEP in placenta) and the decreased frequency and intensity of SEP hits in normal tissues.

Figure 4:

Schematic domain structure of hSEP

Figure 4 shows the putative composition of the domains of hSEP. A globular domain containing Cysteins at the N-terminus is followed by a Prolin rich domain and two cleavage sites (arrows) for serum proteases / serin proteases, e.g. Thrombin, Plasmin or Urokinase. Repetitive units of similar Prolin containing sequences are followed by a Prolin rich domain and a trans-mebrane domain.

Figure 5: Preferred soluble SEP fragments

This Figure shows preferred soluble SEP fragments of the invention.

Figure 6:

Total RNA from mammary gland, and colon tissue was transcribed into cDNA and relative expression of SEP versus 18SrRNA was calculated after quantitative real-time PCR. Absolute expression levels have been analysed by quantitative real-time PCR for a panel of cDNAs from mammary gland and ovary tissue.

Overexpression of SEP was observed in mammary and ovary cancer compared to normal tissue.

Figure 7

Figure 7 describes that HEK 293 cells transfected with SEP produce VEGF.

[redacted]

Examples

Example 1: Isolation of the SEP cDNA by expression screening

An expression screen was conducted in order to isolate novel cDNAs that encode secreted proteins which stimulate endothelial cell proliferation. Plasmid DNAs were prepared on Xantos' proprietary high-throughput robot assembly according to standard Xantos protocols (see WO 03/014346):

Bakteria in growth plates were sedimented by centrifugation and supernatant was exhausted. The pellets were than resuspended with RNAse containing buffer (P1), an alkaline buffer (P2) was added for lysis and afterwards neutralized by an acid buffer (P3).

After a short incubation, plates were again centrifuged and the supernatant transferred into additional plates. To clear the suspension from bacterial endotoxins buffer P4 was added and mixed. The supernatants of an additional centrifugation were than transferred to third plate and mixed with silica to bind plasmid DNA. The silica was washed, therefore the plate was centrifuged and the pellets were resuspended with acetone on a plate shaker. The plates were again centrifuged and the acetone was exhausted and evaporated. The DNA was eluted by mixing the dry silica pellet with water (60°C) and after a centrifugation step the DNA containing supernatant was transferred into the last plate.

For incubation and mixing a plate shaker was used and the buffers were added using an eight channel dispenser.

(P1: Tris EDTA with RNAse, P2: NaOH / SDS, P3: potassium acetate, P4: SDS in isopropanol)

To facilitate the production of the proteins encoded by individual cDNA clones, $2.2x10^4$ 293 HEK cells were seeded in 96-well tissue culture plates (Costar) in 100µl DMEM medium containing 5% FCS (Invitrogen). Transfection of 18000 cDNAs from a clone collection (MGC Clone Collection (IRAK-Collection („Mammalian Gene Collection"; RZPD, Berlin) described in Strausberg RL, Feingold EA, Klausner RD, Collins FS. The Mammalian Gene Collection. Science, 1999, 286, 455-457) on 293 cells was performed 24hrs post seeding using calcium phosphate co-precipitation. Precipitates were removed after 4 hours and cells were switched to nutrient deficient DMEM (DMEM, 1.5%FCS, 1% Na-pyruvate, 1% Glutamine, 100µg/ml gentamycin, 0.5µg/ml amphotericin B). Human umbilical cord vein endothelial cells (HUVEC) were cultured in ECGM with supplements (Promocell Heidelberg, single quots) containing 1 % serum, 50µg/ml gentamycin, 0.4µg/ml amphotericin B and 50U/ml nystatin. HUVECS were plated at 2.5 x 10^3 cells /well on day 3. Before transfer of supernatants on day 4, 90µl of medium was removed, HUVECS were washed once with 200µl of PBS, then 75µl of nutrient deficient medium (ECBM, with supplements, Promocell, Heidelberg) containing 1µg/ml hydrocortisol, 50µg/ml gentamycin, 0.4µg/ml amphotericin B and 50U/ml nystatin was added following 25µl of supernatants from the transfected 293 cells. Supernatants were incubated for 4 days on HUVEC cells. Readout was performed using Alamar Blue (Biosource, California USA). For each well of a 96well plate, 11µl of Alamar Blue reagent were mixed with 9µl of ECBM and the resulting 20µl were added directly to the HUVEC cells without removal of medium. Incubation was performed at 37°C for 4 hours. Alamar Blue fluorescence was measured at 530nm excitation and 590nm emission.

Positive control for proliferation of HUVECs was supernatant containing VEGF derived from the clone collection.

Negative controls were supernatants from vector-transfected cells and PDGF-transfected 293 cells.

This screen led to the isolation of a cDNA which will be referred to as Stimulator of Endothelial Proliferation, SEP. The original SEP clone identified was the IMAGE clone 5123637 derived from a murine liver cDNA library. To identify a

human orthologue for mSEP, BLAST searches against the human UniGene database were performed. They revealed the presence of the mRNA sequence of the hypothetical protein KIAA1271 with a low E-value of about 1e-25. On amino acid level, however, the E-value increases to 5e-125 with an overall homology of 50% between the murine and the human predicted proteins. The assumption that the respective genes may be orthologous is supported by chromosomal localisation studies: the mouse locus of 5123637 is syntenic to the human locus of KIAA1271, 2F2, and 20p13 respectively.

Example 2: Verification of proliferation-inducing activity

For the verification of the proliferation-inducing activity of SEP, mSEP (murine SEP; Xantos clone collection), hSEP (human SEP; received by RZPD clone services) and controls were transfected into HEK293 cells and supernatants were transferred onto HUVEC as described for the screen (Example 1) except that all manipulations were carried out manually. Figure 1 shows the proliferation-inducing activity of mSEP and hSEP in comparison to VEGF.

Example 3: Verification of specific expression

In order to investigate the cell type specificity of SEP supernatants from transfected 293 HEK cells were also added to normal human dermal fibroblasts (NHDF). NHDF were seeded at 1,000 cells per well on 96-well tissue culture plates two days prior to the transfer in 100µl complete Fibroblast Growth Medium (Promocell, Heidelberg). 24h prior to the transfer the medium was changed to 100µl Fibroblast Basal Medium (Promocell, Heidelberg) containing 75µg/ml gentamycin, 50ng/ml amphotericin B. After 25µl of 293 HEK supernatant had been transferred cells were incubated for 4 days and viable cell number was assessed by Alamar Blue reduction as above. Figure 2 demonstrates that mSEP and hSEP

were unable to stimulate NHDF proliferation to levels above empty vector controls. However, the cells were clearly responsive to supernatants containing FGF-2 or PDGF. These results demonstrate that SEP acts specifically on endothelial but not fibroblast cells.

Example 4: Expression analysis of hSEP in comparison to VEGF

Expression analyses of human SEP and VEGF were performed using the Expressed Sequence Tag data provided by the Cancer Genome Anatomy Project of the National Cancer Institute, Bethesda, Maryland, USA. SEP was represented by Unigene Cluster Hs.183669 and VEGF was represented by Hs.73793 of Unigene build Hs.160. EST frequencies per tissue were normalized to 200,000 total EST per tissue. Pooled tissues and tissues for which both the VEGF and SEP frequency were zero were excluded from the analyses. The results are shown in Figure 3.

Example 5: Structure and separate functional domains of SEP

The primary amino acid sequence of SEP (seqID AAH44952.1) forms a protein of 540 amino acids (estimates size 59,4 of kDa), which is anchored to the membrane by a carboxyterminal membrane spanning domain followed by a hydrophilic stop-transfer sequence at the C-terminal end of the molecule. Further details related to the domain structure of SEP are provided in Figure 4. Extracellular domains, which appear to be separated from each other by flexible Gly/Ser rich interdomain linker sequences include repeats which contain 4x multiples of the sequence (L/V)-P-S-K-(LV)-P-T, as well as additional proline rich modules. The amino terminal domain contains multiple cysteins which can form disulfide bonds. Of particular interest is the observation that two very flexible and hence exposed sequence stretches at position 180-2 and 255-8 are preceded by arginine rich se-

[redacted]

quences at position 165-72 and 231-40. Although these sequences are not identified as specific 'classical' consensus sequences for recognition by extracellular or serum proteases per se, they can be considered to provide exposed sensitive sites for proteolytic processing of SEP. A further protease sensitive site may be located directly preceding the C-terminal transmembrane domain at position 509-14. Examples of products of proteolytic processing of SEP by surface-bound or extracellular proteases are represented by sequence ID's 7 to 17.

It has to be noted that the N-terminal protein fragments of SEP, as well as all those that have become separated from the transmembrane domain from the extracellular side, are to be considered as soluble extracellular proteins and peptides. These products can express their biological function at the site of production (highest extracellular concentration) as well as at nearby and remote locations which are different from their side of production.

Example 6: Identification of SEP interacting protein

A) General strategy for the identification of SEP interacting protein

Step 1:

Perform database search and find published interactor. Confirm published interactor by selective knock-out (RNAi) in that cellular assay SEP was defined in.

Step 2:

Prerequisite: Get an antibody against SEP or fuse SEP with another protein/peptide that could be either a reporter gene (e.g. GFP or enzyme or radioactive label or other chemical compound) or immunoprecipitable by an antibody.

The fusions could be checked for maintained binding properties in the original functional assay.

a) A second transfection screen: prepare a cDNA library from a transcriptome compromising the interactor (e.g. the transcriptome of the cell SEP was found functional on). Transfect and over express the cDNA clones individually into an interactor-negative cellular background (this could be checked in advance with the fusion-constructs). Detect labelled cells by visual, enzymatic or physical methods targeted to the fusion-partner of SEP. Gain interactor cDNA from cDNA stock.

b) A co-precipitation approach followed by mass spectrometric analysis of bound partners. Optional: Confirm cellular localisation with labelled ligand: Extract the whole cellular extract or the appropriate cellular compartment by precipitating the interactor with SEP. Precipitation could be performed by immobilisation via SEP specific antibodies or immobilisation of SEP via a fused protein, peptide or chemical label.
[Precipitation of membrane proteins might demand

- Special solubilisation conditions (e.g. detergent concentrations) that have to be changed prior to addition of SEP and immobilisation-compound.
- Cross-linking of SEP and interactor to preserve the interaction.]

The precipitate could be processed in the following ways:

i) Separation on protein gels and blotting (optional: proteolytic cleavage prior to or after electrophoresis). Subsequently mass-spectrometric analysis is performed followed by comparison of peptide data with appropriate mass-spec-databases. In case of no such peptide-map-database entry: sequencing of protein spot or cleavage derived peptides and search in protein

and nucleic acid databases (with derived nucleic acid sequences according to the translation code; e.g. search in EST-databases).

ii) Immunisation of animals with precipitated complex or derived parts of it in order to get antibodies against the putative interactor. These antibodies could serve in reverse immuno-precipitations as tools to show interaction between the respective antigen and SEP.

Step 3:

Perform in vivo and in vitro protein-protein binding studies:

a) Yeast or mammalian two-hybrid assay with SEP as bait and a cDNA library cloned into the corresponding pray-vector. The pray-cDNA library should be derived from cells showing SEP exerted function.

b) Phage display hybridisation with recombinant and labelled SEP

c) Hybridisation of protein chips with recombinant and labelled SEP

Step 4:

a) A second transfection screen: prepare a cDNA library from a transcriptome compromising the interactor (e.g. the transcriptome of the cell SEP was found functional on). Transfect and over express the cDNA clones individually into a cellular background negative for interactor expression and SEP function (this could be checked in advance with the fusion-constructs and antibodies). Detect SEP function / activation in these cells by monitoring SEP induced phenotype (e.g. induction of VEGF). Gain interactor cDNA from cDNA stock.

b) A supernatant screen: prepare a cDNA library from a transcriptome compromising the interactor (e.g. the transcriptome of the cell SEP was found functional on). Transfect and over express the cDNA clones individually into a cellular background potentially negative for interactor expression. Transfer supernatant (containing secreted protein coded by the transfected cDNA) to cells positive for SEP expression. Detect SEP function / activation in these cells by monitoring SEP induced phenotype (e.g. induction of VEGF). Gain interactor cDNA from cDNA stock.

B) Variants of identification of SEP interacting proteins depending on the properties of SEP:

1. Identification of a ligand type SEP interactor
   In this variant the following steps could be performed in parallel or alternatively:

   Step 1, step 2b, step 3a+b+c, step 4a+b

2. Identification of a co-receptor type SEP interactor
   Step 1, step 2b, step 3a+b+c, step 4a

3. Identification of a receptor type SEP interactor

   Step 1, step 2a+b, step 3a+b+c, step 4a

[redacted]

Example 7: Increased expression of SEP in mammary and ovary cancer compared to normal tissue

Figure 3 indicates that EST data show high expression of human SEP in cancer versus normal in most tissues.

Therefore expression levels of SEP in RNAs and cDNAs from human mammary gland (normal and cancer), ovary (normal and cancer) and colon (normal and cancer) were analysed by quantitative real-time PCR.

cDNA was synthesized from 1 µg of total RNA in a volume of 20 µl using random hexamers as primer and AMV ReverseTranscriptase (Roche Diagnostics).

Real-time PCR was carried out using a LightCycler (Roche Diagnostics). Reactions were set up in microcapillary tubes using the following final concentrations: 1 µM each of SEP sense (TCA GGA GCA GGA CAC AGA AC) and SEP antisense (TGG AAG GAG ACA GAT GGA GAC) primers, 3 µM $MgCl_2$, 1x SYBR Greenmaster mix and 0,2 µl of cDNA. Cycling conditions were as follows: denaturation (95° C for 10 min), amplification and quantitation(95°C for 10 s, 56°C for 10 s and 72°C for 13 s, with a single fluorescence measurement at the end of the 72°C for 13 s segment) repeated 45 times. A melting curve program (55–95°C with a heating rate of 0.1 ° C/s and continuous fluorescence measurement) and a cooling step to 40°C followed.

For relative quantification the procedure was repeated for 18S rRNA as reference gene. Data were analyzed using LightCycler analysis software.

In complete agreement with the computer prediction shown in figure 3 we observed higher expression of SEP in mammary and ovarian cancer compared to normal tissue. Also, in agreement with figure 3, colon samples showed a high expression of SEP in tumor as well as in normal tissue. (see figure 6)

Example 8 Induction of VEGF

Induction of VEGF by SEP was measured in an ELISA specific for detection of hVEGF. $2x10^4$ HEK 293 cells were transfected in parallel with 0.28µg of the indicated cDNAs (see Fig. 7) and grown in serum reduced culture medium (1.5% FCS). Concentration of hVEGF in the supernatant was determined 48h after transfection according to the manufacturers protocol (PromoKine - Human VEGF ELISA Kit, PromoCell GmbH, Heidelberg, Germany). The empty vector pCMVSport6 was used as negative control. As positive control cells were transfected with an expression plasmid for hVEGF. Shown are means of 4 independent experiments.

Result: The induction of hVEGF by SEP and/or its murine orthologue is significantly higher compared to the vector control (8 to 13 fold). The concentration of hVEGF in supernatants of SEP transfected cells is similar to cells transfected with the expression plasmid for hVEGF.

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Pro Pro Gly Thr Ser Leu Arg Ser Leu Glu Pro Leu Gln Leu Pro Asp
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Gln Asp Thr Gln Pro Pro Glu Ser Pro Val Glu Asn Ser Glu Gln Leu
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His Gln Glu Gln Glu Pro Glu Leu Gly Gly Ala His Ala Ala Asn Val

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Ala Ser Val Pro Ile Ala Thr Tyr Gly Pro Val Ser Pro Thr Val Ser
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Phe Gln Pro Leu Pro Arg Thr Ala Leu Arg Thr Asn Leu Leu Ser Gly
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Val Thr Val Ser Ala Leu Ser Ala Asp Thr Ser Leu Ser Ser Ser Ser
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Pro Pro Glu Arg Asn Ser Lys Gln Ala Lys Glu Thr Pro Glu Gly Pro
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<213> Homo sapiens

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Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
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Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
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Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln
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Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
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Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
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Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala
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Leu Gln Thr Leu Ser Pro Arg Ala Ile Pro Arg Asn Pro Asp Gly Gly
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Pro Leu Glu Ser Ser Ser Asp Leu Ala Ala Leu Ser Pro Leu Thr Ser
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Ser Gly His Gln Glu Lys Asp Thr Glu Leu Gly Ser Thr His Thr Ala
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Gly Ala Thr Ser Ser Leu Thr Pro Ser Arg Gly Pro Val Ser Pro Ser
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Val Ser Phe Gln Pro Leu Ala Arg Ser Thr Pro Arg Ala Ser Arg Leu
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Ser Ser Pro Gly Leu Ala Ser Ala Gly Ala Ala Glu Gly Lys Gln Gly
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Ala Glu Ser Asp Gln Ala Glu Pro Ile Ile Cys Ser Ser Gly Ala Glu
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Ala Pro Ala Asn Ser Leu Pro Ser Lys Val Pro Thr Thr Leu Met Pro
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Val Asn Thr Val Ala Leu Lys Val Pro Ala Asn Pro Ala Ser Val Ser
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Thr Val Pro Ser Lys Leu Pro Thr Ser Ser Lys Pro Pro Gly Ala Val
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Ser Thr Arg Ala Gly Met Val Pro Ser Lys Val Pro Thr Ser Met Val
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Leu Thr Lys Val Ser Ala Ser Thr Val Pro Thr Asp Gly Ser Ser Arg
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Asn Glu Glu Thr Pro Ala Ala Pro Thr Pro Ala Gly Ala Thr Gly Gly
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Ser Ser Ala Trp Leu Asp Ser Ser Phe Glu Asn Arg Gly Leu Gly Ser
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Glu Leu Ser Lys Pro Gly Val Leu Ala Ser Gln Val Asp Ser Pro Phe
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Ser Gly Cys Phe Glu Asp Leu Ala Ile Ser Ala Ser Thr Ser Leu Gly
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Met Gly Pro Cys His Gly Pro Glu Glu Asn Glu Tyr Lys Ser Glu Gly
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Thr Phe Gly Ile His Val Ala Glu Asn Pro Ser Ile Gln Leu Leu Glu
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Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
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Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
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Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Gln Ser Tyr Gln
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Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu
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Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
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Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
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Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala
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Leu Gln Thr Leu Ser Pro Arg Ala Ile Pro Arg Asn Pro Asp Gly Gly
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Pro Leu Glu Ser Ser Ser Asp Leu Ala Ala Leu Ser Pro Leu Thr Ser
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Ser Gly His Gln Glu Gln Asp Thr Glu Leu Gly Ser Thr His Thr Ala
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Val Ser Phe Gln Pro Leu Ala Arg Ser Thr Pro Arg Ala Ser Arg Leu
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Glu Leu Ser Lys Pro Gly Val Leu Ala Ser Gln Val Asp Ser Pro Phe
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Ser Gly Cys Phe Glu Asp Leu Ala Ile Ser Ala Ser Thr Ser Leu Gly
435 440 445

Met Gly Pro Cys His Gly Pro Glu Glu Asn Glu Tyr Lys Ser Glu Gly
450 455 460

Thr Phe Gly Ile His Val Ala Glu Asn Pro Ser Ile Gln Leu Leu Glu
465 470 475 480

Gly Asn Pro Gly Pro Pro Ala Asp Pro Asp Gly Gly Pro Arg Pro Gln
485 490 495

Ala Asp Arg Lys Phe Gln Glu Arg Glu Val Pro Cys His Arg Pro Ser
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515 520 525

Thr Leu Leu Val Val Leu Tyr Arg Arg Arg Leu His
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<210> 7

<211> 508

<212> PRT

<213> artificial sequence

<220>

<223> fragment

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            20   25   30

Cys Leu Thr Ala Arg Asp Gln Asp Arg Leu Arg Ala Thr Cys Thr Leu
        35   40   45

Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
    50   55   60

Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
65   70   75   80

Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln
85 90 95

Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu
100 105 110

Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
115 120 125

Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
130 135 140

Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala
145 150 155 160

Leu Gln Thr Leu Ser Pro Arg Ala Ile Pro Arg Asn Pro Asp Gly Gly
165 170 175

Pro Leu Glu Ser Ser Ser Asp Leu Ala Ala Leu Ser Pro Leu Thr Ser
180 185 190

Ser Gly His Gln Glu Lys Asp Thr Glu Leu Gly Ser Thr His Thr Ala
195 200 205

Gly Ala Thr Ser Ser Leu Thr Pro Ser Arg Gly Pro Val Ser Pro Ser
210 215 220

Val Ser Phe Gln Pro Leu Ala Arg Ser Thr Pro Arg Ala Ser Arg Leu
225 230 235 240

Pro Gly Pro Thr Gly Ser Val Val Ser Thr Gly Thr Ser Phe Ser Ser
245 250 255

Ser Ser Pro Gly Leu Ala Ser Ala Gly Ala Ala Glu Gly Lys Gln Gly
260 265 270

Ala Glu Ser Asp Gln Ala Pro Ile Ile Cys Ser Ser Gly Ala Glu Ala
275 280 285

Pro Ala Asn Ser Leu Pro Ser Lys Val Pro Thr Thr Leu Met Pro Val
290 295 300

Asn Thr Val Ala Leu Lys Val Pro Ala Asn Pro Ala Ser Val Ser Thr
305 310 315 320

Val Pro Ser Lys Leu Pro Thr Ser Ser Lys Pro Pro Gly Ala Val Pro
325 330 335

Asn Ala Leu Thr Asn Pro Ala Pro Ser Lys Leu Pro Ile Asn Ser Thr

[redacted]

340 345 350

Arg Ala Gly Met Val Pro Ser Lys Val Pro Thr Ser Met Val Leu Thr
355 360 365

Lys Val Ser Ala Ser Thr Val Pro Thr Asp Gly Ser Ser Arg Asn Glu
370 375 380

Glu Thr Pro Ala Ala Pro Thr Pro Ala Gly Ala Thr Gly Gly Ser Ser
385 390 395 400

Ala Trp Leu Asp Ser Ser Phe Glu Asn Arg Gly Leu Gly Ser Glu Leu
405 410 415

Ser Lys Pro Gly Val Leu Ala Ser Gln Val Asp Ser Pro Phe Ser Gly
420 425 430

Cys Phe Glu Asp Leu Ala Ile Ser Ala Ser Thr Ser Leu Gly Met Gly
435 440 445

Pro Cys His Gly Pro Glu Glu Asn Glu Tyr Lys Ser Glu Gly Thr Phe
450 455 460

Gly Ile His Val Ala Glu Asn Pro Ser Ile Gln Leu Leu Glu Gly Asn
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Pro Gly Pro Pro Ala Asp Pro Asp Gly Gly Pro Arg Pro Gln Ala Asp
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<213> artificial sequence

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Cys Leu Thr Ala Arg Asp Gln Asp Arg Leu Arg Ala Thr Cys Thr Leu

35 40 45

Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
50 55 60

Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
65 70 75 80

Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln
85 90 95

Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu
100 105 110

Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
115 120 125

Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
130 135 140

Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala
145 150 155 160

Leu Gln Thr Leu Ser Pro Arg Ala Ile Pro Arg Asn Pro Asp Gly Gly
165 170 175

Pro Leu Glu Ser Ser Ser Asp Leu Ala Ala Leu Ser Pro Leu Thr Ser
180 185 190

Ser Gly His Gln Glu Lys Asp Thr Glu Leu Gly Ser Thr His Thr Ala
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Val Ser Phe Gln Pro Leu Ala Arg Ser Thr Pro Arg Ala Ser Arg
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Cys Leu Thr Ala Arg Asp Gln Asp Arg Leu Arg Ala Thr Cys Thr Leu
35 40 45

Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
50 55 60

Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
65 70 75 80

Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln
85 90 95

Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu
100 105 110

Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
115 120 125

Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
130 135 140

Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala
145 150 155 160

Leu Gln Thr Leu Ser Pro Arg Ala Ile Pro Arg Asn Pro Asp Gly Gly
165 170 175

Pro Leu Glu Ser Ser Ser Asp Leu Ala Ala Leu Ser Pro Leu Thr Ser
180 185 190

Ser Gly His Gln Glu Lys Asp Thr Glu Leu Gly Ser Thr His Thr Ala
195 200 205

Gly Ala Thr Ser Ser Leu Thr Pro Ser Arg Gly Pro Val Ser Pro Ser
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Val Ser Phe Gln Pro Leu Ala Arg Ser Thr Pro Arg
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Cys Leu Thr Ala Arg Asp Gln Asp Arg Leu Arg Ala Thr Cys Thr Leu
35 40 45

Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
50 55 60

Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
65 70 75 80

Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln
85 90 95

Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu

100 105 110

Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
115 120 125

Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
130 135 140

Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala
145 150 155 160

Leu Gln Thr Leu Ser Pro Arg Ala Ile Pro Arg Asn Pro Asp Gly Gly
165 170 175

Pro Leu Glu Ser Ser Ser Asp Leu Ala Ala Leu Ser Pro Leu Thr Ser
180 185 190

Ser Gly His Gln Glu Lys Asp Thr Glu Leu Gly Ser Thr His Thr Ala
195 200 205

Gly Ala Thr Ser Ser Leu Thr Pro Ser Arg Gly Pro Val Ser Pro Ser
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Val Ser Phe Gln Pro Leu Ala Arg
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<211> 171

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<223> Fragment

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Cys Leu Thr Ala Arg Asp Gln Asp Arg Leu Arg Ala Thr Cys Thr Leu
35 40 45

Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
50 55 60

Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu

65 70 75 80

Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln

85 90 95

Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu

100 105 110

Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile

115 120 125

Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val

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Gln Glu Thr Gln Ala Pro Glu Ser Pro Gly Glu Asn Ser Glu Gln Ala

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<211> 167

<212> PRT

<213> artificial sequence

<220>

<223> Fragment

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Cys Leu Thr Ala Arg Asp Gln Asp Arg Leu Arg Ala Thr Cys Thr Leu
35 40 45

Ser Gly Asn Arg Asp Thr Leu Trp His Leu Phe Asn Thr Leu Gln Arg
50 55 60

Arg Pro Gly Trp Val Glu Tyr Phe Ile Ala Ala Leu Arg Gly Cys Glu
65 70 75 80

Leu Val Asp Leu Ala Asp Glu Val Ala Ser Val Tyr Glu Ser Tyr Gln
85 90 95

Pro Arg Thr Ser Asp Arg Pro Pro Asp Pro Leu Glu Pro Pro Ser Leu
100 105 110

Pro Ala Glu Arg Pro Gly Pro Pro Thr Pro Ala Ala Ala His Ser Ile
115 120 125

Pro Tyr Asn Ser Cys Arg Glu Lys Glu Pro Ser Tyr Pro Met Pro Val
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Leu Gln Thr Leu Ser Pro Arg
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<210> 13

<211> 341

<212> PRT

<213> artificial sequence

<220>

<223> Fragment

<400> 13

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            20                  25                  30

Thr Glu Leu Gly Ser Thr His Thr Ala Gly Ala Thr Ser Ser Leu Thr
        35                  40                  45

Pro Ser Arg Gly Pro Val Ser Pro Ser Val Ser Phe Gln Pro Leu Ala
    50                  55                  60

Arg Ser Thr Pro Arg Ala Ser Arg Leu Pro Gly Pro Thr Gly Ser Val
65                  70                  75                  80

Val Ser Thr Gly Thr Ser Phe Ser Ser Ser Ser Pro Gly Leu Ala Ser
                85                  90                  95

Ala Gly Ala Ala Glu Gly Lys Gln Gly Ala Glu Ser Asp Gln Ala Pro
            100                 105                 110

Ile Ile Cys Ser Ser Gly Ala Glu Ala Pro Ala Asn Ser Leu Pro Ser
        115                 120                 125
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Lys Val Pro Thr Thr Leu Met Pro Val Asn Thr Val Ala Leu Lys Val
130 135 140

Pro Ala Asn Pro Ala Ser Val Ser Thr Val Pro Ser Lys Leu Pro Thr
145 150 155 160

Ser Ser Lys Pro Pro Gly Ala Val Pro Asn Ala Leu Thr Asn Pro Ala
165 170 175

Pro Ser Lys Leu Pro Ile Asn Ser Thr Arg Ala Gly Met Val Pro Ser
180 185 190

Lys Val Pro Thr Ser Met Val Leu Thr Lys Val Ser Ala Ser Thr Val
195 200 205

Pro Thr Asp Gly Ser Ser Arg Asn Glu Glu Thr Pro Ala Ala Pro Thr
210 215 220

Pro Ala Gly Ala Thr Gly Gly Ser Ser Ala Trp Leu Asp Ser Ser Phe
225 230 235 240

Glu Asn Arg Gly Leu Gly Ser Glu Leu Ser Lys Pro Gly Val Leu Ala
245 250 255

Ser Gln Val Asp Ser Pro Phe Ser Gly Cys Phe Glu Asp Leu Ala Ile

260 265 270

Ser Ala Ser Thr Ser Leu Gly Met Gly Pro Cys His Gly Pro Glu Glu
275 280 285

Asn Glu Tyr Lys Ser Glu Gly Thr Phe Gly Ile His Val Ala Glu Asn
290 295 300

Pro Ser Ile Gln Leu Leu Glu Gly Asn Pro Gly Pro Pro Ala Asp Pro
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Val Pro Cys His Arg
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<210> 14

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<212> PRT

<213> artificial sequence

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20 25 30

Ser Thr His Thr Ala Gly Ala Thr Ser Ser Leu Thr Pro Ser Arg Gly
35 40 45

Pro Val Ser Pro Ser Val Ser Phe Gln Pro Leu Ala Arg Ser Thr Pro
50 55 60

Arg Ala Ser Arg Leu Pro Gly Pro Thr Gly Ser Val Val Ser Thr Gly
65 70 75 80

Thr Ser Phe Ser Ser Ser Ser Pro Gly Leu Ala Ser Ala Gly Ala Ala
85 90 95

Glu Gly Lys Gln Gly Ala Glu Ser Asp Gln Ala Pro Ile Ile Cys Ser
100 105 110

Ser Gly Ala Glu Ala Pro Ala Asn Ser Leu Pro Ser Lys Val Pro Thr

115 120 125

Thr Leu Met Pro Val Asn Thr Val Ala Leu Lys Val Pro Ala Asn Pro
130 135 140

Ala Ser Val Ser Thr Val Pro Ser Lys Leu Pro Thr Ser Ser Lys Pro
145 150 155 160

Pro Gly Ala Val Pro Asn Ala Leu Thr Asn Pro Ala Pro Ser Lys Leu
165 170 175

Pro Ile Asn Ser Thr Arg Ala Gly Met Val Pro Ser Lys Val Pro Thr
180 185 190

Ser Met Val Leu Thr Lys Val Ser Ala Ser Thr Val Pro Thr Asp Gly
195 200 205

Ser Ser Arg Asn Glu Glu Thr Pro Ala Ala Pro Thr Pro Ala Gly Ala
210 215 220

Thr Gly Gly Ser Ser Ala Trp Leu Asp Ser Ser Phe Glu Asn Arg Gly
225 230 235 240

Leu Gly Ser Glu Leu Ser Lys Pro Gly Val Leu Ala Ser Gln Val Asp
245 250 255

Ser Pro Phe Ser Gly Cys Phe Glu Asp Leu Ala Ile Ser Ala Ser Thr
260 265 270

Ser Leu Gly Met Gly Pro Cys His Gly Pro Glu Glu Asn Glu Tyr Lys
275 280 285

Ser Glu Gly Thr Phe Gly Ile His Val Ala Glu Asn Pro Ser Ile Gln
290 295 300

Leu Leu Glu Gly Asn Pro Gly Pro Pro Ala Asp Pro Asp Gly Gly Pro
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Arg Pro Gln Ala Asp Arg Lys Phe Gln Glu Arg Glu Val Pro Cys His
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Arg

<210> 15

<211> 276

<212> PRT

<213> artificial sequence

<220>

<223> Fragment

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20 25 30

Gly Ala Ala Glu Gly Lys Gln Gly Ala Glu Ser Asp Gln Ala Pro Ile
35 40 45

Ile Cys Ser Ser Gly Ala Glu Ala Pro Ala Asn Ser Leu Pro Ser Lys
50 55 60

Val Pro Thr Thr Leu Met Pro Val Asn Thr Val Ala Leu Lys Val Pro
65 70 75 80

Ala Asn Pro Ala Ser Val Ser Thr Val Pro Ser Lys Leu Pro Thr Ser
85 90 95

Ser Lys Pro Pro Gly Ala Val Pro Asn Ala Leu Thr Asn Pro Ala Pro

100 105 110

Ser Lys Leu Pro Ile Asn Ser Thr Arg Ala Gly Met Val Pro Ser Lys
115 120 125

Val Pro Thr Ser Met Val Leu Thr Lys Val Ser Ala Ser Thr Val Pro
130 135 140

Thr Asp Gly Ser Ser Arg Asn Glu Glu Thr Pro Ala Ala Pro Thr Pro
145 150 155 160

Ala Gly Ala Thr Gly Gly Ser Ser Ala Trp Leu Asp Ser Ser Phe Glu
165 170 175

Asn Arg Gly Leu Gly Ser Glu Leu Ser Lys Pro Gly Val Leu Ala Ser
180 185 190

Gln Val Asp Ser Pro Phe Ser Gly Cys Phe Glu Asp Leu Ala Ile Ser
195 200 205

Ala Ser Thr Ser Leu Gly Met Gly Pro Cys His Gly Pro Glu Glu Asn
210 215 220

Glu Tyr Lys Ser Glu Gly Thr Phe Gly Ile His Val Ala Glu Asn Pro
225 230 235 240

Ser Ile Gln Leu Leu Glu Gly Asn Pro Gly Pro Pro Ala Asp Pro Asp
245 250 255

Gly Gly Pro Arg Pro Gln Ala Asp Arg Lys Phe Gln Glu Arg Glu Val
260 265 270

Pro Cys His Arg
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<211> 272

<212> PRT

<213> artificial sequence

<220>

<223> Fragment

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Ser Phe Ser Ser Ser Ser Pro Gly Leu Ala Ser Ala Gly Ala Ala Glu

20 25 30

Gly Lys Gln Gly Ala Glu Ser Asp Gln Ala Pro Ile Ile Cys Ser Ser
35 40 45

Gly Ala Glu Ala Pro Ala Asn Ser Leu Pro Ser Lys Val Pro Thr Thr
50 55 60

Leu Met Pro Val Asn Thr Val Ala Leu Lys Val Pro Ala Asp Pro Ala
65 70 75 80

Ser Val Ser Thr Val Pro Ser Lys Leu Pro Thr Ser Ser Lys Pro Pro
85 90 95

Gly Ala Val Pro Asn Ala Leu Thr Asn Pro Ala Pro Ser Lys Leu Pro
100 105 110

Ile Asn Ser Thr Arg Ala Gly Met Val Pro Ser Lys Val Pro Thr Ser
115 120 125

Met Val Leu Thr Lys Val Ser Ala Ser Thr Val Pro Thr Asp Gly Ser
130 135 140

Ser Arg Asn Glu Glu Thr Pro Ala Ala Pro Thr Pro Ala Gly Ala Thr
145 150 155 160

Gly Gly Ser Ser Ala Trp Leu Asp Ser Ser Phe Glu Asn Arg Gly Leu
165 170 175

Gly Ser Glu Leu Ser Lys Pro Gly Val Leu Ala Ser Gln Val Asp Ser
180 185 190

Pro Phe Ser Gly Cys Phe Glu Asp Leu Ala Ile Ser Ala Ser Thr Ser
195 200 205

Leu Gly Met Gly Pro Cys His Gly Pro Glu Glu Asn Glu Tyr Lys Ser
210 215 220

Glu Gly Thr Phe Gly Ile His Val Ala Glu Asn Pro Ser Ile Gln Leu
225 230 235 240

Leu Glu Gly Asn Pro Gly Pro Pro Ala Asp Pro Asp Gly Gly Pro Arg
245 250 255

Pro Gln Ala Asp Arg Lys Phe Gln Glu Arg Glu Val Pro Cys His Arg
260 265 270

<210> 17

<211> 269

<212> PRT

<213> Artificial

<400> 17

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            20   25   30

Gly Ala Glu Ser Asp Gln Ala Pro Ile Ile Cys Ser Ser Gly Ala Glu
        35   40   45

Ala Pro Ala Asn Ser Leu Pro Ser Lys Val Pro Thr Thr Leu Met Pro
    50   55   60

Val Asn Thr Val Ala Leu Lys Val Pro Ala Asn Pro Ala Ser Val Ser
65   70   75   80

Thr Val Pro Ser Lys Leu Pro Thr Ser Ser Lys Pro Pro Gly Ala Val
                85   90   95

Pro Asn Ala Leu Thr Asn Pro Ala Pro Ser Lys Leu Pro Ile Asn Ser
100 105 110

Thr Arg Ala Gly Met Val Pro Ser Lys Val Pro Thr Ser Met Val Leu
115 120 125

Thr Lys Val Ser Ala Ser Thr Val Pro Thr Asp Gly Ser Ser Arg Asn
130 135 140

Glu Glu Thr Pro Ala Ala Pro Thr Pro Ala Gly Ala Thr Gly Gly Ser
145 150 155 160

Ser Ala Trp Leu Asp Ser Ser Phe Glu Asn Arg Gly Leu Gly Ser Glu
165 170 175

Leu Ser Lys Pro Gly Val Leu Ala Ser Gln Val Asp Ser Pro Phe Ser
180 185 190

Gly Cys Phe Glu Asp Leu Ala Ile Ser Ala Ser Thr Ser Leu Gly Met
195 200 205

Gly Pro Cys His Gly Pro Glu Glu Asn Glu Tyr Lys Ser Glu Gly Thr
210 215 220

Phe Gly Ile His Val Ala Glu Asn Pro Ser Ile Gln Leu Leu Glu Gly

225 230 235 240

Asn Pro Gly Pro Pro Ala Asp Pro Asp Gly Gly Pro Arg Pro Gln Ala

245 250 255

Asp Arg Lys Phe Gln Glu Arg Glu Val Pro Cys His Arg

260 265

Summary

The present invention relates to a novel angiogenic factor, SEP, as well as to soluble derivatives thereof and to their use in pharmaceutical or diagnostic compositions.

June 10, 2003
X62263USPRO BÖ/FLZ/bto

Xantos Biomedicine AG

Claims

1. A soluble SEP (sSEP) or a functional active soluble derivative thereof.

2. The derivative of claim 1, wherein the derivative exhibits a sequence homology of at least 25 % to the sSEP.

3. The sSEP or functional derivative thereof of any of claims 1 or 2, being devoid of a transmembrane domain of SEP or of a functional active variant thereof.

4. The sSEP or functional derivative thereof of any of claims 1 to 3, having a C-terminal amino acid corresponding to amino acid 510, 249, 246, 242, 171 or 167 of SEP according to SEQ ID NO: 4 or having a C-terminal amino acid corresponding to the equivalent amino acid of a sSEP derivative.

5. A pharmaceutical composition, comprising

   a) the sSEP or derivative thereof of any of claims 1 to 4,
   b) SEP as defined in SEQ ID NO: 2, 4 or 6,
   c) a functional active derivative of the SEP of section b), and /or
   d) a nucleic acid encoding the molecules of section a), b) or c),

   optionally in combination with a pharmaceutically acceptable carrier.

6. The pharmaceutical composition of claim 5, further comprising VEGF and/or a functional derivative thereof.

7. The sSEP or derivative thereof of any of claims 1 to 4, SEP as defined in SEQ ID NO: 2, 4 or 6 or a functional active derivative thereof, and/or a nucleic acid encoding these molecules for use in therapy.

8. Use of

   a) the sSEP or derivative thereof of any of claims 1 to 4,
   b) SEP as defined in SEQ ID NO: 2, 4 or 6,
   c) a functional active derivative of the SEP of section b), and /or
   d) a nucleic acid encoding the molecules of sections a), b) or c),

   for the preparation of a pharmaceutical composition for the treatment of ischemic or dental diseases, smoker's leg and diabetic ulcers, for the stimulation of wound healing or for the amelioration or preservation of infertility.

9. The use of claim 8, in combination with VEGF and/or a functional active derivative thereof.

10. A diagnostic agent comprising

   a) the sSEP or derivative thereof of any of claims 1 to 4,
   b) SEP as defined in SEQ ID NO: 2, 4 or 6,
   c) a functional active derivative of the SEP of section b),
   d) a nucleic acid encoding the molecules of sections a), b) or c), and /or
   e) means for the detection of the molecules of sections a), b), c) or d)

11. The sSEP or derivative thereof of any of claims 1 to 4, SEP as defined in SEQ ID NO: 2, 4 or 6 or a functional active derivative thereof, a nucleic acid encoding these proteins and/or means for the detection of these proteins or nucleic acids for use in therapy.

12. Use of

a) the sSEP or derivative thereof of any of claims 1 to 4,
b) SEP as defined in SEQ ID NO: 2, 4 or 6,
c) a functional active derivative of the SEP of section b),
d) a nucleic acid encoding the molecules of sections a), b) or c), and /or
e) means for the detection of the molecules of sections a), b) , c) or d)

for the preparation of a diagnostic agent for the diagnosis of tumors and/or tumor progression.

13. An inhibitor of the sSEP or derivative thereof of any of claims 1 to 4 or of the SEP as defined in SEQ ID NO: 2, 4 or 6 or of a functional active derivative thereof.

14. The inhibitor of claim 13, selected from the group consisting of antibodies, antisense oligonucleotides, siRNA, Low molecular weight molecules (LMWs) and SEP receptor antagonists.

15. A pharmaceutical composition, comprising the inhibitor of any of claims 13 or 14, optionally in combination with a pharmaceutically acceptable carrier.

16. The pharmaceutical composition of claim 15, further comprising a VEGF inhibitor.

17. The inhibitor of any of claims 13 or 14, for use in therapy.

18. Use of an inhibitor of any of claims 13 or 14 for the preparation of a pharmaceutical composition for the treatment of cancer, rheumatoid arthritis, psoriasis, artherosclerosis, retinopathy, osteoarthritis, endometriosis and chronic inflammation.

19. The use of claim 18, wherein the cancer is selected from the group consisting of brain cancer, pancreas carcinoma, stomach cancer, colon carcinoma, skin cancer, especially melanoma, bone cancer, kidney carcinoma, liver cancer, lung carcinoma, ovary cancer, mamma carcinoma, uterus carcinoma, prostate cancer and testis carcinoma.

20. The use of any of claims 18 or 19, in combination with a VEGF inhibitor.

21. A method for the identification of a SEP inhibitor, wherein a potential inhibitor is tested for its activity to block the effects of SEP or of a functional derivative thereof.

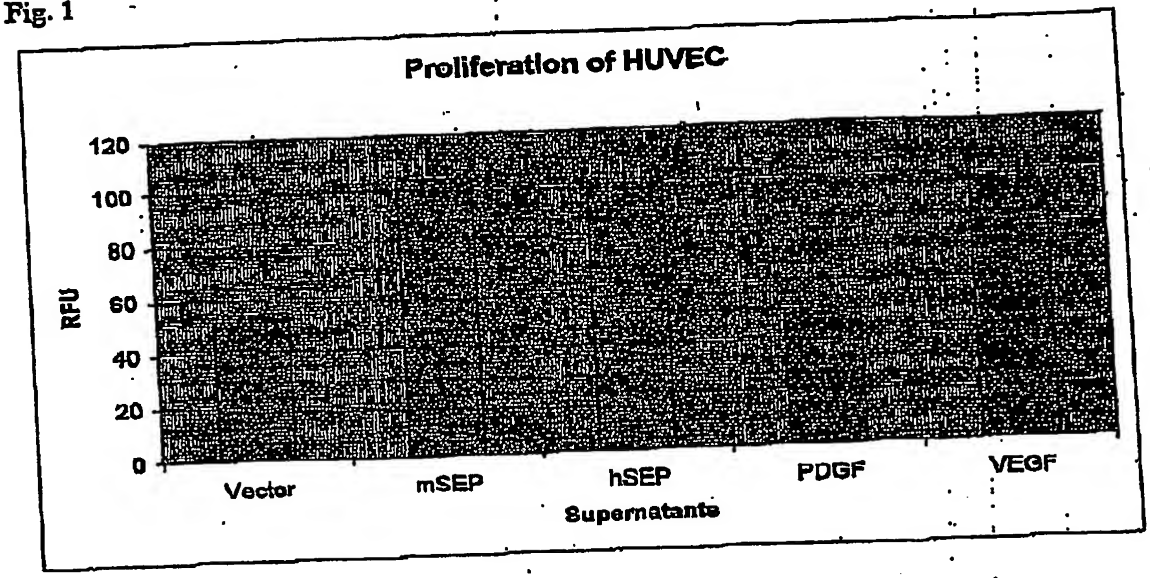
22. A method for the preparation of a pharmaceutical composition, wherein a SEP inhibitor is identified according to claim 21, synthesized in adequate amounts and finally formulated into a pharmaceutical composition.

23. Use of SEP, sSEP or a derivative thereof for the identification of proteins that bind or interact with SEP, wherein

a) a potential SEP interactor is brought into contact with SEP or a functional derivative thereof, and

b) binding of the potential interactor to SEP or the functional derivative thereof is determined.

Fig. 1


Proliferation of HUVEC
RFU
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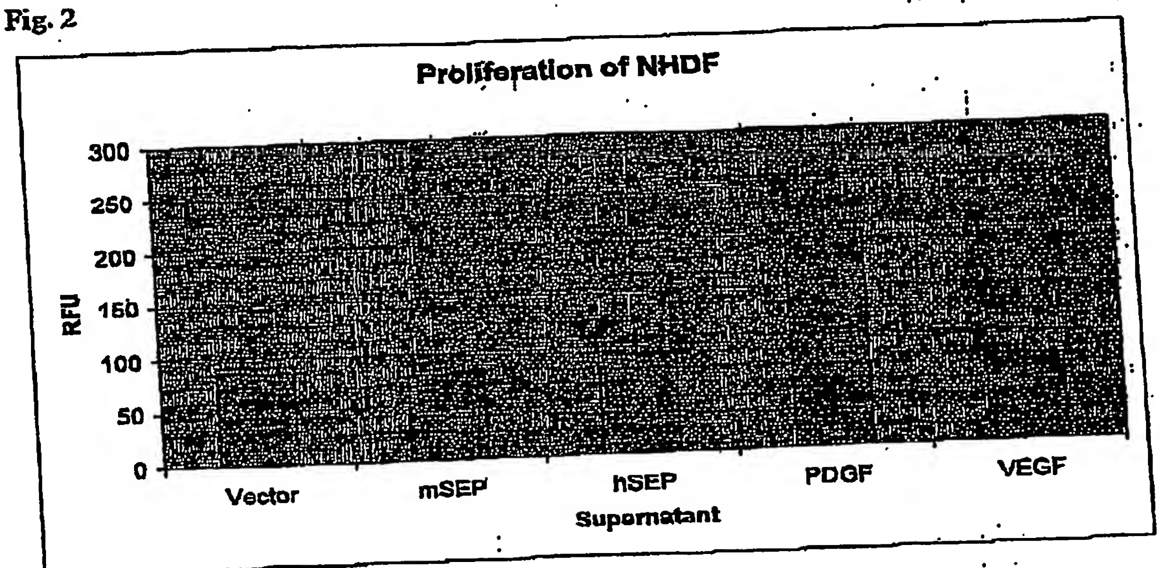
20
40
60
80
100
120
Vector
mSEP
hSEP
PDGF
VEGF
Supernatants

Fig. 2


Proliferation of NHDF
RFU
0
50
100
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Vector
mSEP
hSEP
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VEGF
Supernatant

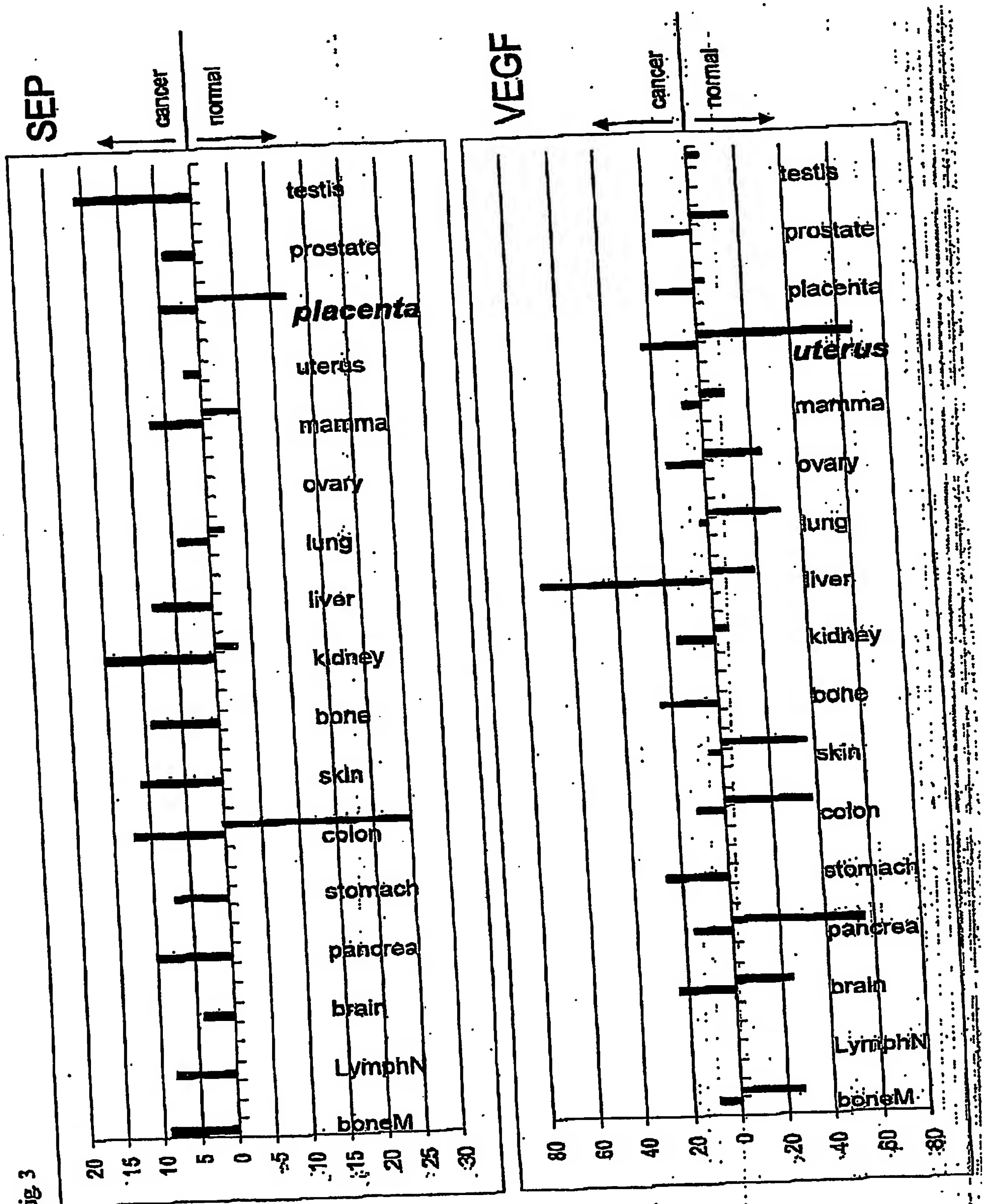
SEP
cancer
normal
testis
prostate
placenta
uterus
mamma
ovary
lung
liver
kidney
bone
skin
colon
stomach
pancrea
brain
LymphN
boneM
20 15 10 5 0 -5 -10 -15 -20 -25 -30
VEGF
cancer
normal
testis
prostate
placenta
uterus
mamma
ovary
lung
liver
kidney
bone
skin
colon
stomach
pancrea
brain
LymphN
boneM
80 60 40 20 0 -20 -40 -60 -80

Fig 3

Fig. 4

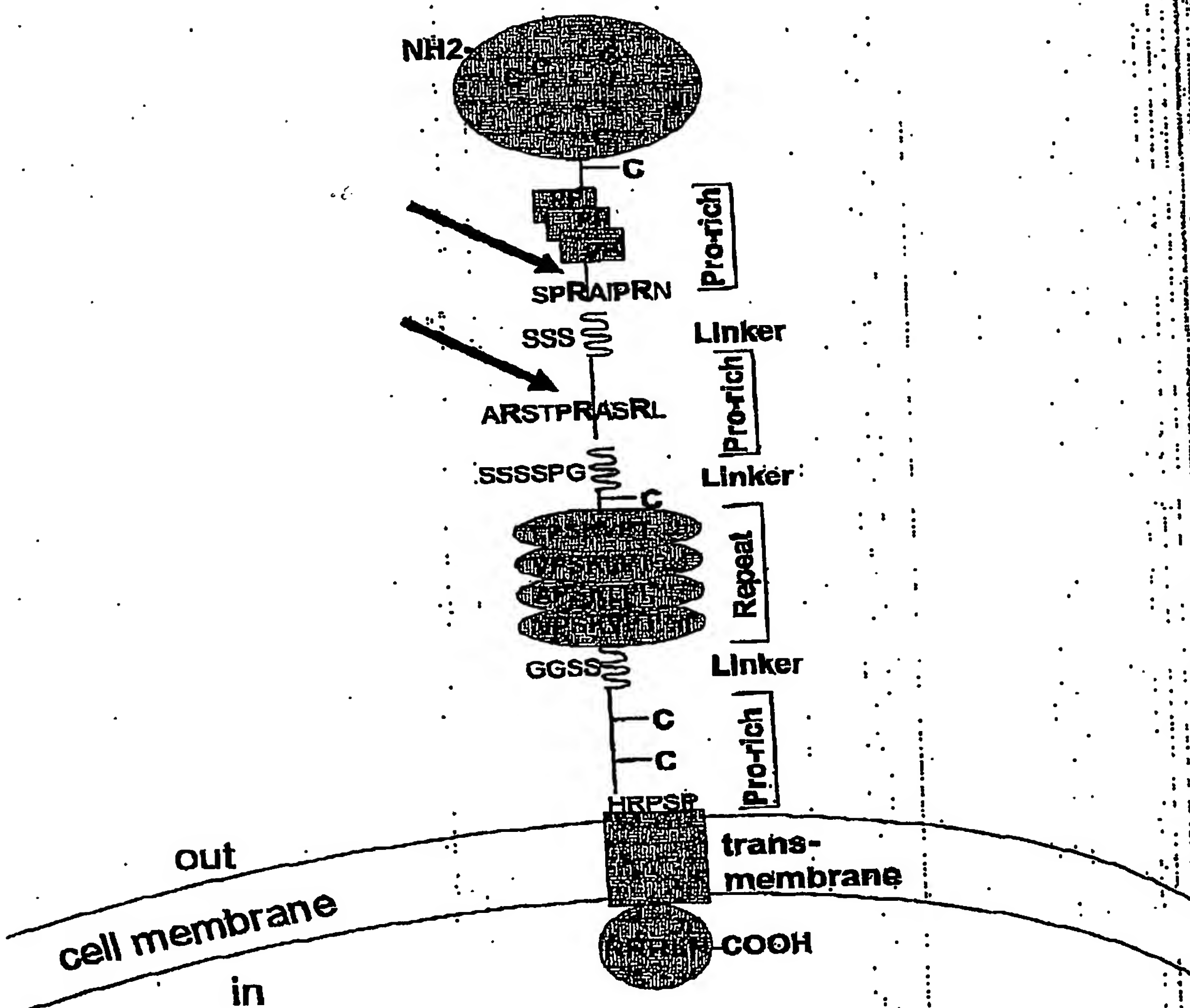
NH2-
C
Pro-rich
SPRAIPRN
SSS
Linker
ARSTPRASRL
Pro-rich
SSSSPG
Linker
C
Repeat
GGSS
Linker
C
C
Pro-rich
HRPSP
trans-
membrane
out
cell membrane
in
COOH

Fig. 5

Fragment 1 (1-510):

MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRLRATCTLSGNR
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PDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAPESPG
ENSEQALQTLSPRAIPRNPDGGPLESSSDLAALSPLTSSGHQEKDTELGSTH
TAGATSSLTPSRGPVSPSVSFQPLARSTPRASRLPGPTGSVVSTGTSFSSSS
PGLASAGAAEGKQGAESDQAPIICSSGAEAPANSLPSKVPTTLMPVNTVALK
VPANPASVSTVPSKLPTSSKPPGAVPNALTNPAPSKLPINSTRAGMVPSKVP
TSMVLTKVSASTVPTDGSSRNEETPAAPTPAGATGGSSAWLDSSFENRGLG
SELSKPGVLASQVDSPFSGCFEDLAISASTSLGMGPCHGPEENEYKSEGTF
GIHVAENPSIQLLEGNPGPPADPDGGPRPQADRKFQEREVPCHR

Fragment 2 (1-249):

MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRLRATCTLSGNR
DTLWHLFNTLQRRPGWVEYFIAALRGCELVDLADEVASVYESYQPRTSDRP
PDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAPESPG
ENSEQALQTLSPRAIPRNPDGGPLESSSDLAALSPLTSSGHQEKDTELGSTH
TAGATSSLTPSRGPVSPSVSFQPLARSTPRASR

Fragment 3 (1-246):

MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRLRATCTLSGNR
DTLWHLFNTLQRRPGWVEYFIAALRGCELVDLADEVASVYESYQPRTSDRP
PDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAPESPG
ENSEQALQTLSPRAIPRNPDGGPLESSSDLAALSPLTSSGHQEKDTELGSTH
TAGATSSLTPSRGPVSPSVSFQPLARSTPR

Fragment 4 (1-242):

MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRLRATCTLSGNR
DTLWHLFNTLQRRPGWVEYFIAALRGCELVDLADEVASVYESYQPRTSDRP
PDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAPESPG
ENSEQALQTLSPRAIPRNPDGGPLESSSDLAALSPLTSSGHQEKDTELGSTH
TAGATSSLTPSRGPVSPSVSFQPLAR

Fragment 5 (1-171):

MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRLRATCTLSGNR
DTLWHLFNTLQRRPGWVEYFIAALRGCELVDLADEVASVYESYQPRTSDRP
PDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAPESPG
ENSEQALQTLSPRAIPR

Fragment 6 (1-167):

MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRLRATCTLSGNR
DTLWHLFNTLQRRPGWVEYFIAALRGCELVDLADEVASVYESYQPRTSDRP
PDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAPESPG
ENSEQALQTLSPR

Fragment 7 (168-510):

AIPRNPDGGPLESSSDLAALSPLTSSGHQEKDTELGSTHTAGATSSLTPSRG
PVSPSVSFQPLARSTPRASRLPGPTGSVVSTGTSFSSSSPGLASAGAAEGK
QGAESDQAPIICSSGAEAPANSLPSKVPTTLMPVNTVALKVPANPASVSTVP
SKLPTSSKPPGAVPNALTNPAPSKLPINSTRAGMVPSKVPTSMVLTKVSAST
VPTDGSSRNEETPAAPTPAGATGGSSAWLDSSFENRGLGSELSKPGVLASQ
VDSPFSGCFEDLAISASTSLGMGPCHGPEENEYKSEGTFGIHVAENPSIQLLE
GNPGPPADPDGGPRPQADRKFQEREVPCHR

Fragment 8 (172-510):

NPDGGPLESSSDLAALSPLTSSGHQEKDTELGSTHTAGATSSLTPSRGPVSP
SVSFQPLARSTPRASRLPGPTGSVVSTGTSFSSSSPGLASAGAAEGKQGAE
SDQAPIICSSGAEAPANSLPSKVPTTLMPVNTVALKVPANPASVSTVPSKLPT
SSKPPGAVPNALTNPAPSKLPINSTRAGMVPSKVPTSMVLTKVSASTVPTDG
SSRNEETPAAPTPAGATGGSSAWLDSSFENRGLGSELSKPGVLASQVDSPF
SGCFEDLAISASTSLGMGPCHGPEENEYKSEGTFGIHVAENPSIQLLEGNPG
PPADPDGGPRPQADRKFQEREVPCHR

Fragment 9 (243-510):

STPRASRLPGPTGSVVSTGTSFSSSSPGLASAGAAEGKQGAESDQAPIICSS
GAEAPANSLPSKVPTTLMPVNTVALKVPANPASVSTVPSKLPTSSKPPGAVP
NALTNPAPSKLPINSTRAGMVPSKVPTSMVLTKVSASTVPTDGSSRNEETPA
APTPAGATGGSSAWLDSSFENRGLGSELSKPGVLASQVDSPFSGCFEDLAI
SASTSLGMGPCHGPEENEYKSEGTFGIHVAENPSIQLLEGNPGPPADPDGG
PRPQADRKFQEREVPCHR

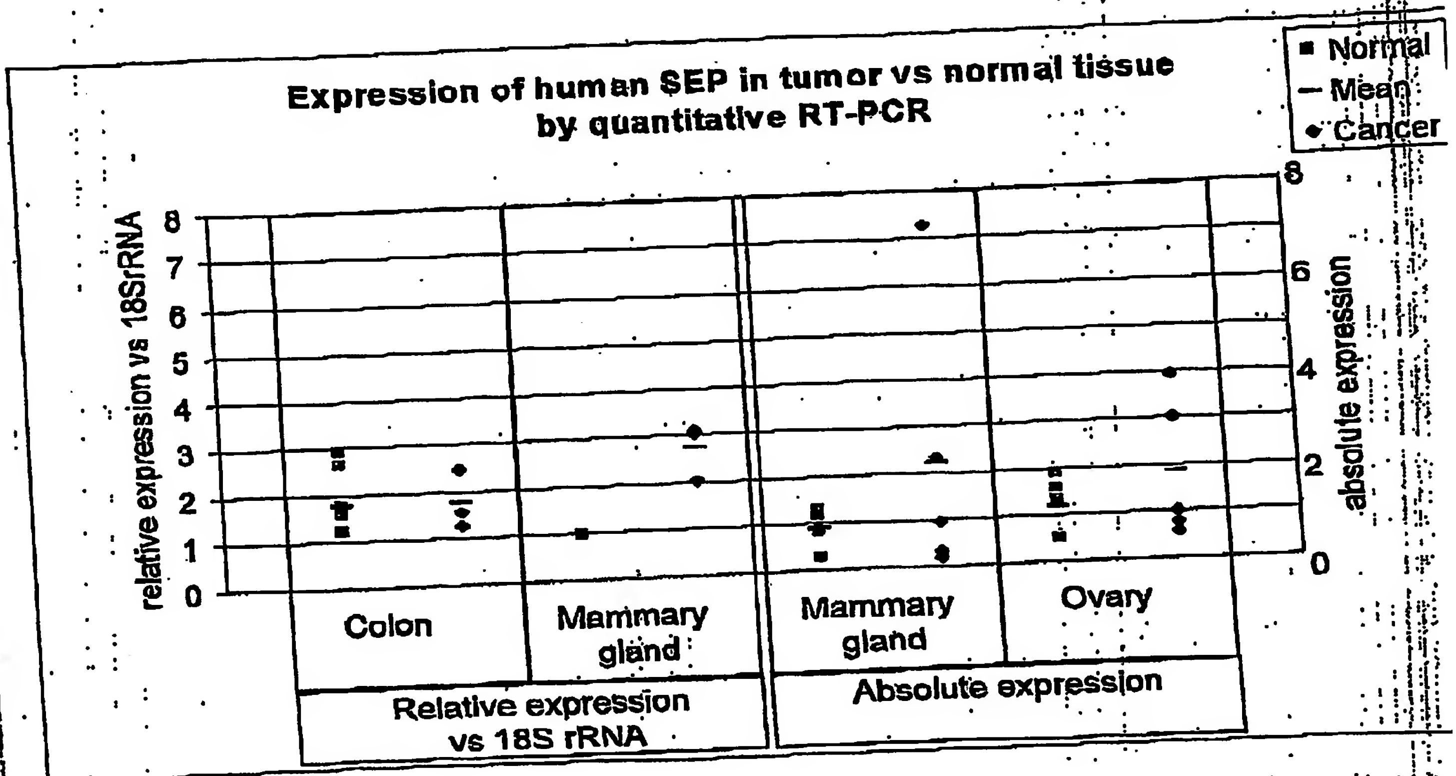
Fragment 10 (247-510):

ASRLPGPTGSVVSTGTSFSSSSPGLASAGAAEGKQGAESDQAPIICSSGAEA
PANSLPSKVPTTLMPVNTVALKVPANPASVSTVPSKLPTSSKPPGAVPNALT
NPAPSKLPINSTRAGMVPSKVPTSMVLTKVSASTVPTDGSSRNEETPAAPTP
AGATGGSSAWLDSSFENRGLGSELSKPGVLASQVDSPFSGCFEDLAISAST
SLGMGPCHGPEENEYKSEGTFGIHVAENPSIQLLEGNPGPPADPDGGPRPQ
ADRKFQEREVPCHR

Fragment 11 (250-510):

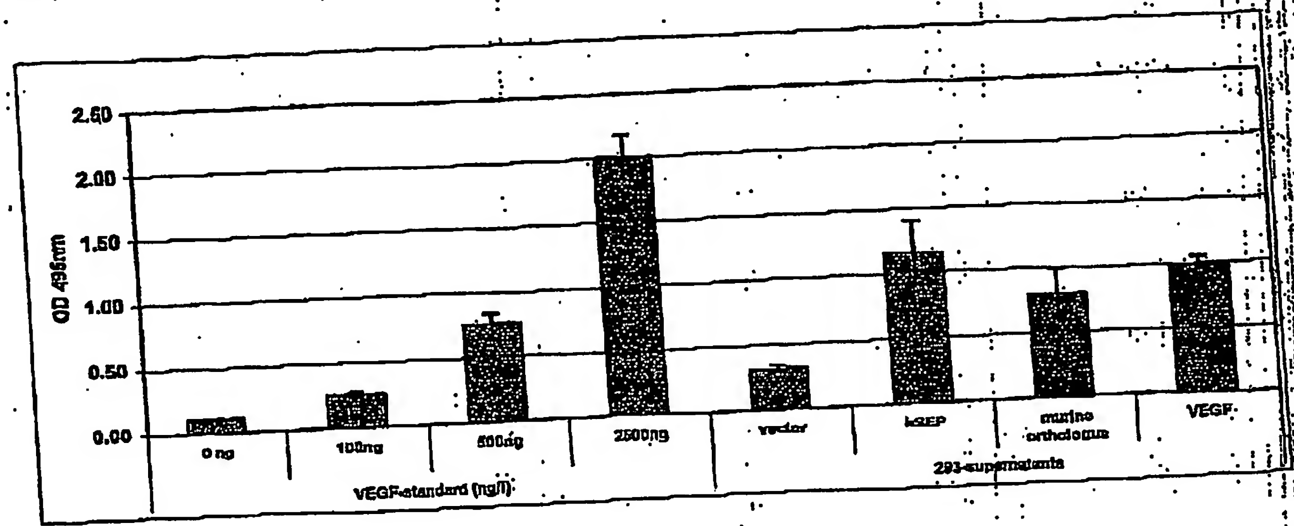
LPGPTGSVVSTGTSFSSSSPGLASAGAAEGKQGAESDQAPIICSSGAEAPAN
SLPSKVPTTLMPVNTVALKVPANPASVSTVPSKLPTSSKPPGAVPNALTNPA
PSKLPINSTRAGMVPSKVPTSMVLTKVSASTVPTDGSSRNEETPAAPTPAGA
TGGSSAWLDSSFENRGLGSELSKPGVLASQVDSPFSGCFEDLAISASTSLG
MGPCHGPEENEYKSEGTFGIHVAENPSIQLLEGNPGPPADPDGGPRPQADR
KFQEREVPCHR

Fig. 6



Expression of human SEP in tumor vs normal tissue by quantitative RT-PCR
Normal
Mean
Cancer
relative expression vs 18SrRNA
absolute expression
8
7
6
5
4
3
2
1
0
8
6
4
2
0
Colon
Mammary gland
Mammary gland
Ovary
Relative expression vs 18S rRNA
Absolute expression

Fig. 7


2.50
2.00
1.50
1.00
0.50
0.00
OD 495nm
0 ng
100ng
500ng
2500ng
vector
murine
orthologue
VEGF
VEGF-standard (ng/l)
293-supernatants